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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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For: METHOD OF MODULATING CELL SURVIVAL AND REAGENTS USEFUL FOR SAME

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Sir:

Enclosed are three certified foreign priority documents that are being submitted for filing in the captioned application. These are: Australian Provisional Patent Application No. PP 6351; Australian Provisional Patent Application No. PQ 0701; and Australian Provisional Patent Application No. PP 6353.

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Respectfully submitted,
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I, JONNE YABSLEY, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PP 6351 for a patent by THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH filed on 07 October 1998.

WITNESS my hand this
Tenth day of April 2001

J R Yabsley

JONNE YABSLEY
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Regulation 3.2

The Walter and Eliza Hall Institute of Medical Research

AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION

for the invention entitled:

"A METHOD OF MODULATING CELL SURVIVAL AND REAGENTS USEFUL FOR SAME-II"

The invention is described in the following statement:

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A METHOD OF MODULATING CELL SURVIVAL AND REAGENTS USEFUL FOR SAME-II

FIELD OF THE INVENTION

The present invention relates generally to a method for modulating cell survival. Modulation of cell survival includes inducing, enhancing or otherwise promoting cell survival such as the survival of neuronal cells as well as facilitating cell death such as the death of targetted cancer cells. The modulation of cell survival is mediated by a region identified on the p75 neurotrophin receptor ($p75^{NTR}$) required for death signalling. The present invention further provides genetic molecules which encode the death signalling region of $p75^{NTR}$ which are useful in antagonising death signal function as well as promoting cell death when expressed in targetted cells. The present invention also contemplates recombinant peptides, polypeptides and proteins as well as chemical equivalents, derivatives and homologues thereof which comprise the death signalling portion of $p75^{NTR}$.

BACKGROUND OF THE INVENTION

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description.

The increasing sophistication of recombinant DNA technology is greatly facilitating research and development in the medical and allied health fields. This is particularly the case in the development of recombinant cytokines and growth factors for use in the treatment of diabetes, aquired immunodeficency syndrome (AIDS) and a number of cancers.

However, despite this developing knowledge of cytokine and growth factor effector molecules, their full exploitation requires an understanding of the corresponding cellular receptors and the complex biochemical and physiological signalling pathways initiated following interaction with ligands or following other stimulation such as disease, receptor aggregation or trauma.

A number of soluble trophic factors have been shown to exhibit an effect on neuronal survival *in vivo*. Many of these factors act directly on the developing neuron within, for example, the dorsal root ganglia (DRG). One factor of particular importance is nerve growth factor (NGF) [1]. The p75 neurotrophin receptor (hereinafter referred to as "$p75^{NTR}$"), which is capable of complexing with trk growth factor receptors, is required for high affinity NGF binding and survival signalling. Although NGF has been proposed as a potential therapeutic molecule to promote survival of neurons, NGF is a multifunctional molecule and its pleiotrophy may adversely effect a range of non-neuronal cells.

$p75^{NTR}$ is also multifunctional. It has now been shown that $p75^{NTR}$ is capable of acting as a death receptor. Elevated $p75^{NTR}$ expression results in increased cell death *in vitro* and *in vivo* [2-4]. Furthermore, down-regulation of $p75^{NTR}$ prevents neuronal death after growth-factor withdrawal or axotomy [5, 6]. Consistent with the dual functions of $p75^{NTR}$, mice with deleted $p75^{NTR}$ genes have a dramatic reduction of NGF dependent neurons, such as dorsal root ganglia, but increased numbers of other neuron populations (sympathetic and basal forebrain neurons) suggesting lack of naturally occuring cell death [7, 8]. $p75^{NTR}$ is also implicated in mediating death of neuronal, oligodendrocytes and Schwann cells [8, 9].

$p75^{NTR}$ is a member of the tumor necrosis factor (TNF) receptor/Fas superfamily, showing homology not only to the extracellular ligand binding domain but also to a cytoplasmic motif known as the "death domain", so termed because of the cytotoxic actions of proteins containing the domain [9].

There is an accumulating body of evidence which suggests that $p75^{NTR}$ is involved in mediating cell death in a variety of degenerative diseases. During adulthood, $p75^{NTR}$ expression is down-regulated in most brain areas but is rapidly induced in ischemia (stroke) and results in transient increased $p75^{NTR}$ expression and apoptosis, as do both peripheral and motor nerve lesions [10-12]. $p75^{NTR}$ is also up regulated in patients with MND [13], and in experimental allergic encephalomyelitis (a model of multiple sclerosis; [14]). Intriguingly, in the basal forebrain and hippocampus, areas involved in learning and memnory, $p75^{NTR}$ is highly expressed in aged rodents and in Alzheimer's patients, where extensive neuronal death

is occuring [15, 16]. These data suggest that p75^{NTR} is involved not only in normal developmental cell death, but may mediate the cell death occuring after injury or in neurodegenerative disease.

In work leading up to the present invention, the inventors sought to elucidate the region on p75^{NTR} which mediates death signalling. The inventors surprisingly determined that the death signal is not the cytoplasmic motif known as the death domain [9] but is a region adjacent the membrane domain on p75^{NTR}. The identification of this region provides for an opportunity to modulate cell survival by antagonising the death signalling region or promoting apoptosis by providing cells with the genetic material to express the death signalling region adjacent, proximal or otherwise juxtaposed or associated with the membrane or to express the death signalling region in multimeric form.

SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

One aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides or complementary sequence of nucleotides which encode an amino acid sequence which is capable of signalling, inducing or otherwise facilitating the death of a cell in which said amino acid sequence is adjacent, proximal or otherwise juxtaposed to the membrane of said cell or said amino acid sequence is in multimeric form.

Another aspect of the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides or complementary sequence of nucleotides which encodes a peptide, polypeptide or protein capable of signalling, inducing or otherwise facilitating death of a cell

in which it is expressed wherein said peptide, polypeptide or protein comprises a membrane associating portion and/or a multimer-forming portion and a portion which corresponds to all or part of the cytoplasmic region of $p75^{NTR}$ or a functional equivalent, derivative or homologue thereof.

Yet another aspect of the present invention contemplates homologues, analogues and derivatives of a nucleic acid molecule which encodes a peptide, polypeptide or protein which is capable of signalling inducing or otherwise facilitating death of a cell in which it is expressed wherein said peptide, polypeptide or protein comprises a membrane associating portion and/or a multimer-forming portion and a portion which corresponds to all or part of the cytoplasmic region of $p75^{NTR}$ or a functional equivalent, derivative or homologue thereof.

Still another aspect of the present invention provides a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence which is substantially as set forth in SEQ ID NO:3 or is a nucleotide sequence capable of hybridising thereto under low stringency conditions at 42 °C or is a nucleotide sequence having at least 60% identity thereto.

Still yet another aspect of the present invention contemplates a nucleic acid molecule comprising a nucleotide sequence or a complementary form thereof, which nucleotide sequence encodes an amino acid sequence substantially as set forth in SEQ ID NO:4 or a derivative, homologue or chemical equivalent thereof or an amino acid sequence having at least 60% identity thereto.

Even yet another aspect of the present invention provides a genetic construct comprising an isolated nucleic acid molecule which comprises a sequence of nucleotides which corresponds or is complementary to a death signal region from $p75^{NTR}$ or a homologue, analogue or derivative thereof.

Another aspect of the present invention contemplates an isolated peptide, polypeptide or protein comprising the cytoplasmic region of $p75^{NTR}$ which signals, induces or otherwise

facilitates cell death when said peptide, polypeptide or protein is adjacent, proximal or otherwise juxtaposed to a membrane-associating region such as from $p75^{NTR}$ or other membrane molecule and/or said peptide, polypeptide or protein is capable of forming multimers or a derivative, homologue, chemical equivalent or analogue of said peptide, polypeptide or protein. This aspect of the present invention does not extend to the full length $p75^{NTR}$.

Still another aspect of the present invention contemplates a method for inhibiting, reducing or otherwise antagonising a $p75^{NTR}$-mediated death signal in a neuronal cell, said method comprising introducing a nucleic acid molecule capable of being expressed to an expression product which corresponds to a non-membrane associated form of the $p75^{NTR}$ death signal region or a derivative, functional equivalent or homologue thereof.

Yet another aspect of the invention contemplates a method for inhibiting, reducing or otherwise antagonising a $p75^{NTR}$-mediated death signal in a neuronal cell, said method comprising contacting a cell carrying a $p75^{NTR}$ with a death signal-inhibiting effective amount of a molecule capable of antagonising the death signal of $p75^{NTR}$ or a component of the death signalling pathway.

Even still another aspect of the present invention provides a biological composition comprising a genetic molecule capable of being expressed into a $p75^{NTR}$ death signal antagonist or a $p75^{NTR}$ death signal.

Another aspect of the present invention is directed to a biological composition comprising a molecule capable of antagonising $p75^{NTR}$-mediated death signalling of a cell.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a diagrammatic representation showing plasmid constructs with and without the death signalling region. The black region is the putative "death domain" [9] but which is not directly involved in $p75^{NTR}$ mediated cell death.

Figure 2 is a graphical representation showing survival of DRG neurons 17 hours after microinfection and cultured in LIF. The data show that the amino acid domain juxtaposed to the membrane is required for death signalling rather than the putative "death domain" [9].

Figure 3 is a graphical representation showing DRG survival 16 hours after microinjection and cultured in LIF. The data show that over 90% of cells die when expressing the death signal linked to the membrane.

Figure 4 is a graphical representation showing DRG survival 20 hours after microinjection and cultured in LIF. These data show that when the death signal is not associated with the membrane, that the ability to induce death is removed.

Figure 5 is a graphical representation showing that the C35 protein (i.e. $p75^{NTR}$ death signal region) inhibits death signalling mediated by $p75^{NTR}$.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention arose in part following an investigation of the neurotrophin receptor, $p75^{NTR}$, in its capacity as a death signalling protein. Although the $p75^{NTR}$ molecule comprises a putative death domain [9], in accordance with the present invention, this death domain is not directly associated with $p75^{NTR}$-mediated cell death. Rather, a region adjacent, proximal or otherwise juxtaposed to the membrane domain of $p75^{NTR}$ is required for cell death.

Accordingly, one aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides or complementary sequence of nucleotides which encode an amino acid sequence which is capable of signalling, inducing or otherwise facilitating the death of a cell in which said amino acid sequence is adjacent, proximal or otherwise juxtaposed to the membrane of said cell or said amino acid sequence is in multimeric form.

Reference herein to the signalling, inducing or otherwise facilitating the death of a cell or a death signal is meant to be construed in its broadest sense meaning that the amino acid

sequence plays a role in a pathway leading to cell death. The death signal may also be regarded as an apoptopic signal. Although not wishing to limit the present invention to any one theory or mode of action, it is proposed herein that there is a pathway from $p75^{NTR}$ activation to caspase activation and cellular degeneration. It is further proposed that $p75^{NTR}$-mediated cell death may occur directly or indirectly *via* Bcl-2.

The present specification refers interchangably to death signal, death signal region, signalling, inducing or otherwise facilitating the death of a cell and C35.

The nucleic acid molecule of the present invention may encode a non-full length $p75^{NTR}$ molecule although to facilitate cell death, the nucleic acid molecule must encode all or part of the cytoplasmic portion of the $p75^{NTR}$ molecule and a sufficient amount of the membrane domain such that the region referred to herein as the death signal is membrane associated. A "part" of the cytoplasmic domain of $p75^{NTR}$ includes all or a death-inducing functional part of a 35 amino acid region juxtaposed to the membrane domain. Alternatively, the cytoplasmin domain of the $p75^{NTR}$ molecule is in multimeric form or capable of forming multimers. A multimer comprises two or more copies of the molecule such as a dimer, trimer or larger copy molecule.

The term "membrane associated" means that the death signal is adjacent, proximal or otherwise juxtaposed to the membrane of a cell expressing the nucleic acid molecule.

The "death signal region" and other related terms are used herein to describe functionally the region of the cytoplasmic portion of $p75^{NTR}$ which is adjacent, proximal or otherwise juxtaposed to a region of $p75^{NTR}$ which associates with the membrane or which cytoplasmic portion is in multimeric form. The death signal region is not the same portion of the molecules as the "death domain" [9] although there may be functional similarities in death signalling.

Accordingly, another aspect of the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides or complementary sequence of nucleotides which

encodes a peptide, polypeptide or protein capable of signalling, inducing or otherwise facilitating death of a cell in which it is expressed wherein said peptide, polypeptide or protein comprises a membrane associating portion and/or a multimer-forming portion and a portion which corresponds to all or part of the cytoplasmic region of $p75^{NTR}$ or a functional equivalent, derivative or homologue thereof.

In order to signal, induce or otherwise facilitate death of a cell, the death signal region is preferably adjacent, proximal or otherwise juxtaposed to the cell membrane. However, the present invention extends to multimeric forms and attachments which facilitate same. A multimer comprises two or more molecules.

In one embodiment, the membrane portion is derived from $p75^{NTR}$ or a functional equivalent, derivative or homologue thereof. In another embodiment, the membrane domain is from another molecule such as a receptor or other ligand-binding molecule. Examples of receptors according to this aspect of the present invention include cytokine receptors (e.g. the Leukaemia Inhibitory Factor (LIF) receptor, interleukin receptor, and colony-stimulating factor receptors). Examples of ligand-binding molecules include immunoglobulins and T cell receptors.

When in multimeric form, the molecule is only optionally associated with the membrane to effect cell death.

The nucleic acid molecule may comprise cDNA or genomic DNA or may comprise ribonucleotides such as mRNA. The nucleic acid molecule may be derived from a cDNA or genomic molecule encoding $p75^{NTR}$ or a derivative or homologue thereof or may be prepared by the stepwise addition of nucleotides in a defined sequence.

The nucleic acid molecule of the present invention may also be considered as corresponding to a "gene".

Reference herein to a "gene" is to be taken in its broadest context and includes:

(i) a classical genomic gene consisting of transcriptional and/or translational regulatory sequences and/or a coding region and/or non-translated sequences (i.e. introns, 5′- and 3′- untranslated sequences);

(ii) mRNA or cDNA corresponding to the coding regions (i.e. exons) optionally comprising 5′- or 3′-untranslated sequences of the gene; or

(iii) an amplified DNA fragment or other recombinant nucleic acid molecule produced *in vitro* and comprising all or a part of the coding region and/or 5′- or 3′-untranslated sequences of the gene.

The term "gene" is also used to describe synthetic or fusion molecules encoding all or part of a functional product. A functional product is one which comprises a sequence of nucleotides or is complementary to a sequence of nucleotides which encodes a functional death signal from $p75^{NTR}$ or its derivative or homologue.

The nucleotide sequence of the present invention may correspond to the cDNA or genomic sequence of a gene encoding $p75^{NTR}$ or a death signal region thereof or may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or additions. Nucleotide insertional derivatives of the nucleic acid molecule of the present invention include 5′ and 3′ terminal fusions as well as intra-sequence insertions of single or multiple nucleotides. Insertional nucleotide sequence variants are those in which one or more nucleotides are introduced into a predetermined site in the nucleotide sequence although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterised by the removal of one or more nucleotides from the sequence. Substitutional nucleotide variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide inserted in its place. Such a substitution may be "silent" in that the substitution does not change the amino acid defined by the codon. Alternatively, substituents are designed to alter one amino acid for another similar acting amino acid, or amino acid of like charge, polarity, or hydrophobicity.

Accordingly, another aspect of the present invention contemplates homologues, analogues and derivatives of a nucleic acid molecule which encodes a peptide, polypeptide or protein which is capable of signalling, inducing or otherwise facilitating death of a cell in which it is expressed wherein said peptide, polypeptide or protein comprises a membrane associating portion and/or multimer-forming portion and a portion which corresponds to all or part of the cytoplasmic region of $p75^{NTR}$ or a functional equivalent, derivative or homologue thereof.

For the present purpose, "homologues" of a nucleic acid molecule as herein defined or of a nucleotide sequence shall be taken to refer to an isolated nucleic acid molecule which is substantially the same as the nucleic acid molecule of the present invention or its complementary nucleotide sequence, notwithstanding the occurrence within said sequence, of one or more nucleotide substitutions, insertions, deletions, or rearrangements.

"Analogues" of a nucleic acid molecule as herein defined or of a nucleotide sequence set forth herein shall be taken to refer to an isolated nucleic acid molecule which is substantially the same as a nucleic acid molecule of the present invention or its complementary nucleotide sequence, notwithstanding the occurrence of any non-nucleotide constituents not normally present in said isolated nucleic acid molecule, for example carbohydrates, radiochemicals including radionucleotides, reporter molecules such as, but not limited to DIG, alkaline phosphatase or horseradish peroxidase, amongst others.

"Derivatives" of a nucleic acid molecule as herein defined or of a nucleotide sequence set forth herein shall be taken to refer to any isolated nucleic acid molecule which contains significant sequence similarity to said sequence or a part thereof. Generally, the nucleotide sequence of the present invention may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or insertions. Nucleotide insertional derivatives of the nucleotide sequence of the present invention include 5′ and 3′ terminal fusions as well as intra-sequence insertions of single or multiple nucleotides or nucleotide analogues. Insertional nucleotide sequence variants are those in which one or more nucleotides or nucleotide analogues are introduced into a predetermined site in the nucleotide sequence of said sequence, although random insertion is also possible with suitable screening

of the resulting product being performed. Deletional variants are characterised by the removal of one or more nucleotides from the nucleotide sequence. Substitutional nucleotide variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide or nucleotide analogue inserted in its place.

In one embodiment, the derivatives encode a peptide, polypeptide or protein which induces cell death. In another embodiment, the derivatives do not induce cell death but antagonise the death signal.

The nucleic acid molecule of the present invention may be based on a nucleotide sequence of the gene or cDNA encoding $p75^{NTR}$ from any animal such as from mammals. Preferred mammals include humans, primates, livestock animals (e.g. cows, sheep, horses, pigs, donkeys, goats), laboratory test animals (e.g. rabbits, mice, rats, guinea pigs, hamsters), companion animals (e.g. dogs, cats) and captive wild animals.

A particularly preferred sequence is from human or primate or murine $p75^{NTR}$.

The present invention is exemplified using a nucleotide sequence from rat $p75^{NTR}$ cDNA. This is done, however, with the understanding that the nucleotide sequence may be from $p75^{NTR}$ genomic or cDNA from any animal.

Accordingly, another aspect of the present invention provides a nucleic acid molecule comprising a nucleotide sequence or a complementary form thereof wherein said nucleotide sequence is capable of hybridising to SEQ ID NO:1 under low stringency conditions at 42 °C.

The nucleotide sequence set forth in SEQ ID NO:1 is the cDNA sequence encoding $p75^{NTR}$. The nucleic acid molecule according to this aspect of the present invention does not extend to the full length $p75^{NTR}$ cDNA sequence but comprises a portion which encodes an amino acid sequence which signals, induces or otherwise facilitates cell death when associated with a membrane portion of $p75^{NTR}$ or other molecules.

Accordingly, another aspect of the present invention provides a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence which is substantially as set forth in SEQ ID NO:7 or is a nucleotide sequence capable of hybridising thereto under low stringency conditions at 42 °C or is a nucleotide sequence having at least 60% identity thereto.

The nucleotide sequence set forth in SEQ ID NO:7 is the death signal defined herein associated with $p75^{NTR}$. This sequence encodes a 35 amino acid region also referred to herein as "C35".

Reference herein to a low stringency at 42°C includes and encompasses from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for hybridisation, and at least about 1M to at least about 2M salt for washing conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5M to at least about 0.9M salt for hybridisation, and at least about 0.5M to at least about 0.9M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01M to at least about 0.15M salt for hybridisation, and at least about 0.01M to at least about 0.15M salt for washing conditions.

The present invention further contemplates a nucleic acid molecule comprising a nucleotide sequence or a complementary form thereof, which nucleotide sequence encodes an amino acid sequence substantially as set forth in SEQ ID NO:8 or a derivative, homologue or chemical equivalent thereof or an amino acid sequence having at least 60% identity thereto.

The amino acid sequence of SEQ ID NO:8 corresponds to the amino acid sequence of the $p75^{NTR}$ death signal.

The term "identity" as used herein includes exact identity between compared sequences at the nucleotide or amino acid level. Where there is non-identity at the nucleotide level, the term

"similarity" may also be used and includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. In a particularly preferred embodiment, nucleotide and sequence comparisons are made at the level of identity rather than similarity. Any number of programs are available to compare nucleotide and amino acid sequences. Preferred programs have regard to an appropriate alignment. One such program is Gap which considers all possible alignment and gap positions and creates an alignment with the largest number of matched bases and the fewest gaps. Gap uses the alignment method of Needleman and Wunsch [17]. Gap reads a scoring matrix that contains values for every possible GCG symbol match. GAP is available on ANGIS (Australian National Genomic Information Service) at website http://mel1.angis.org.au..

The present invention further comprises a nucleic acid molecule comprising the nucleotide sequence:

$$\{n_1 - - - n_x\}_b \text{ a } \{n'_1 - - - n'_y\}_c \text{ a } \{n''_1 - - - n''_z\}_d$$

wherein

$\{n_1 - - - n_x\}$ is a sequence of x nucleotides encoding an extracellular portion of a receptor or ligand-binding molecule;

$\{n'_1 - - - n'_y\}$ is a sequence of y nucleotides encoding a transmembrane peptide, polypeptide or protein or a molecule capable of inducing multimerisation;

$\{n''_1 - - - n''_z\}$ is a sequence of z nucleotides comprising a nucleotide sequence substantially as set forth in SEQ ID NO:7 or a nucleotide sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:8 or a nucleotide sequence capable of hybridising thereto under low stringency conditions at 42 °C or a nucleotide sequence having at least 60% identity to SEQ ID NO:7;

b, c and d may be the same or difference and each is 0, 1 or >1;

x, y and z may be the same or different and each is 0, 1 or >1;

a is a nucleotide bond;

wherein when c is 1 or >1 and d is 1 or >1 and wherein when the molecule is expressed in a neuronal cell, the expression product signals, induces or otherwise facilitates cell death.

Preferably, $\{n_1 - - - n_x\}$ comprises the nucleotide sequence substantially as set forth in SEQ ID NO:3 or is a nucleotide sequence having at least about 60% identity thereto or is capable of hybridising thereto under low stringency conditions at 42 °C.

Preferably, $\{n'_1 - - - n'_y\}$ comprises the nucleotide sequence substantially as set forth in SEQ ID NO:5 or is a nucleotide sequence having at least about 60% identity thereto or is capable of hybridising thereto under low stringency conditions at 42 °C.

The nucleotide sequences $\{n_1 - - - n_x\}$, $\{n'_1 - - - n'_y\}$ and $\{n''_1 - - - n''_z\}$ may be in any order and in any combination.

For the production of a recombinant peptide, polypeptide or protein comprising the death signal, the nucleic acid molecule of the present invention is placed, in the sense orientation, in operable connection with a suitable promoter sequence and introduced into a suitable expression system, for example a bacterial, yeast, baculovirus, plant, animal or other expression system.

Accordingly, a further aspect of the present invention provides a genetic construct comprising an isolated nucleic acid molecule which comprises a sequence of nucleotides which corresponds or is complementary to a death signal region from $p75^{NTR}$ or a homologue, analogue or derivative thereof.

According to this embodiment, the coding region of the death signal from $p75^{NTR}$ may be placed in operable connection with a promoter sequence such that a gene product is capable of being expressed under the control of said promoter sequence.

Optionally, said genetic construct further comprises a terminator sequence.

In the present context, the term "in operable connection with" is used to indicate that expression of the isolated nucleotide sequence is under the control of the promoter sequence with which it is connected.

The term "terminator" refers to a DNA sequence at the end of a transcriptional unit which signals termination of transcription. Terminators are 3'-non-translated DNA sequences containing a polyadenylation signal, which facilitates the addition of polyadenylate sequences to the 3'-end of a primary transcript. Terminators active in plant cells are known and described in the literature. They may be isolated from bacteria, fungi, viruses, animals and/or plants.

Examples of terminators particularly suitable for use in the genetic constructs of the present invention include the SV40 polyadenylation signal, amongst others.

Reference herein to a "promoter" is to be taken in its broadest context and includes the transcriptional regulatory sequences of a classical genomic gene, including the TATA box which is required for accurate transcription initiation in eukaryotic cells, with or without a CCAAT box sequence and additional regulatory elements (i.e. upstream activating sequences, enhancers and silencers). For expression in prokaryotic cells, such as bacteria, the promoter should at least contain the -35 box and -10 box sequences.

A promoter is usually, but not necessarily, positioned upstream or 5', of the nucleotide sequence encoding the death signal of $p75^{NTR}$, the expression of which it regulates. Furthermore, the regulatory elements comprising a promoter are usually positioned within 2 kb of the start site of transcription of the gene.

In the present context, the term "promoter" is also used to describe a synthetic or fusion molecule, or derivative which confers, activates or enhances expression of an isolated nucleic acid molecule, in a cell, such as a plant, animal, insect, fungal, yeast or bacterial cell. Preferred promoters may contain additional copies of one or more specific regulatory elements, to further enhance expression of a nucleic acid molecule which expression it regulates and/or to alter the spatial expression and/or temporal expression of same. For example, regulatory elements which confer copper inducibility may be placed adjacent to a heterologous promoter sequence driving expression of a nucleic acid molecule, thereby conferring copper inducibility on the expression of said molecule.

Placing an isolated nucleic acid molecule under the regulatory control of a promoter sequence means positioning said molecule such that expression is controlled by the promoter sequence. Promoters are generally positioned 5′ (upstream) to the genes that they control. In the construction of heterologous promoter/structural gene combinations it is generally preferred to position the promoter at a distance from the gene transcription start site that is approximately the same as the distance between that promoter and the gene it controls in its natural setting, i.e., the gene from which the promoter is derived. As is known in the art, some variation in this distance can be accommodated without loss of promoter function. Similarly, the preferred positioning of a regulatory sequence element with respect to a heterologous gene to be placed under its control is defined by the positioning of the element in its natural setting, i.e., the genes from which it is derived. Again, as is known in the art, some variation in this distance can also occur.

Examples of promoters suitable for use in genetic constructs of the present invention include viral, fungal, bacterial, animal and plant derived promoters capable of functioning in plant, animal, insect, fungal, yeast or bacterial cells. The promoter may regulate the expression of the nucleic acid molecule constitutively, or differentially with respect to the tissue in which expression occurs or, with respect to the developmental stage at which expression occurs, or in response to external stimuli such as physiological stresses, or plant pathogens, or metal ions, amongst others.

Preferably, the promoter is capable of regulating expression of a nucleic acid molecule in a yeast or bacterial cell.

Examples of preferred promoters include the bacteriophage T7 promoter, bacteriophage T3 promoter, SP6 promoter, *lac* promoter, *tac* promoter, SV40 early promoter, and the like.

The genetic construct contemplated herein is introduced into a suitable expression system for a time and under conditions sufficient for expression of said death signal from $p75^{NTR}$ to occur.

The genetic construct may also comprising a nucleotide sequence corresponding to all or part of the membrane domain of $p75^{NTR}$ or other membrane molecules.

Accordingly, a further aspect of the invention contemplates a recombinant peptide, polypeptide or protein produced by expressing the isolated nucleic acid molecule herein described in a suitable host cell. The present invention extends also to a synthetic peptide fragment of said recombinant gene product.

The present invention further contemplates an isolated peptide, polypeptide or protein comprising the cytoplasmic region of $p75^{NTR}$ which signals, induces or otherwise facilitates cell death when said peptide, polypeptide or protein is adjacent, proximal or otherwise juxtaposed to a membrane-associating region such as from $p75^{NTR}$ or other membrane molecule and/or is in multimeric form or a derivative, homologue, chemical equivalent or analogue of said peptide, polypeptide or protein. This aspect of the present invention does not extend to the full length $p75^{NTR}$.

Suitable molecules according to this aspect of the present invention include a peptide, polypeptide or protein corresponding to a soluble form of the death signalling region of $p75^{NTR}$ or a molecule capable of antagonising that region or a component of the death signalling pathway. An example of a component of the death signalling pathway is Bcl-2.

The peptide, polypeptide or protein of this aspect of the present invention is useful *inter alia* as a therapeutic molecule to antagonise $p75^{NTR}$ -mediated death signalling. For example, the peptide, polypeptide or protein may themselves be administered to directly antagonise $p75^{NTR}$-mediated death signalling or the peptide, polypeptide or protein may need to be chemically modified to facilitate penetration into the cell. Alternatively, the death signalling region of $p75^{NTR}$ may be used to screen for antagonists of this region. Such antagonists may, for example, be identified following natural product screening or the screening of chemical libraries. For natural product screening suitable environments include, but are not limited to, plants, bacteria and other microorganisms, river and sea beds, coral and arctic or antarctic regions. The present invention also contemplates antagonists directed to other components of the $p75^{NTR}$-mediated death signalling pathway. Such components to be targeted include but are not limited to Bcl-2 or related or homologous molecules.

Preferably, the peptide, polypeptide or protein comprises an amino acid sequence substantially as set forth in SEQ ID NO:8 or an amino acid sequence having at least 60% identity thereto or a chemical equivalent, derivative, homologue or analogue of said peptide, polypeptide or protein.

The term "isolated" means that the peptide, polypeptide or protein of the present invention is provided in a form which is distinct from that which occurs in nature, preferably wherein one or more contaminants have been removed. Accordingly, the isolated peptide, polypeptide or protein of the invention may be partially-purified or substantially pure, in which a substantial amount of the contaminants have been removed or in sequencably pure or substantially homogeneous form.

The term "sequencably pure" means that the isolated peptide, polypeptide or protein is provided in a form which is sufficiently purified to facilitate amino acid sequence determination using procedures known to those skilled in the art.

The term "substantially homogeneous" means that the isolated peptide, polypeptide or protein of the present invention is at least about 95% free of contaminants, more preferably at

least about 99% free of contaminants, including 100% purity.

The present invention extends to a range of derivatives and chemical analogues of the peptide, polypeptide or protein.

Furthermore, the amino acids of a homologous polypeptide may be replaced by other amino acids having similar properties, for example hydrophobicity, hydrophilicity, hydrophobic moment, charge or antigenicity, and so on.

"Analogues" encompass death signal containing peptides, polypeptides or proteins which are at least about 60% identical to the $p75^{NTR}$ death signal sequence (SEQ ID NO:8), notwithstanding the occurrence of any non-naturally occurring amino acid analogues therein. "Analogues" also encompass polypeptide mimotypes.

The term "derivative" in relation to a peptide, polypeptide or protein shall be taken to refer hereinafter to mutants, parts or fragments derived from the functional $p75^{NTR}$ molecule or death signal region thereof or derivatives thereof which may or may not possess the death signal activity of the functional $p75^{NTR}$. Derivatives include modified peptides in which ligands are attached to one or more of the amino acid residues contained therein, such as carbohydrates, enzymes, proteins, polypeptides or reporter molecules such as radionuclides or fluorescent compounds. Glycosylated, fluorescent, acylated or alkylated forms of the subject peptides are particularly contemplated by the present invention. Additionally, derivatives of the peptide, polypeptide or protein described herein comprise fragments or parts of an amino acid sequence disclosed herein are within the scope of the invention, as are homopolymers or heteropolymers comprising two or more copies of the subject polypeptides. Procedures for derivatizing peptides are well-known in the art.

A homologue, analogue or derivative of SEQ ID NO:2 or SEQ ID NO:8 may comprise an amino acid substitution or said SEQ ID NO: 2 or 8 may encompass amino acid alterations in which an amino acid is replaced with a different naturally-occurring or a non-conventional amino acid residue. Such substitutions may be classified as "conservative", in which case an

amino acid residue contained in a phospholipase inhibitory protein is replaced with another naturally-occurring amino acid of similar character, for example Gly↔Ala, Val↔Ile↔Leu, Asp↔Glu, Lys↔Arg, Asn↔Gln or Phe↔Trp↔Tyr.

Substitutions encompassed by the present invention may also be "non-conservative", in which an amino acid residue which is present in a phospholipase inhibitory protein is substituted with an amino acid having different properties, such as a naturally-occurring amino acid from a different group (eg. substituted a charged or hydrophobic amino acid with alanine), or alternatively, in which a naturally-occurring amino acid is substituted with a non-conventional amino acid.

Amino acid substitutions are typically of single residues, but may be of multiple residues, either clustered or dispersed.

Naturally-occurring amino acids include those listed in Table 1. Non-conventional amino acids encompassed by the invention include, but are not limited to those listed in Table 2.

Amino acid deletions will usually be of the order of about 1-10 amino acid residues, while insertions may be of any length. Deletions and insertions may be made to the N-terminus, the C-terminus or be internal deletions or insertions. Generally, insertions within the amino acid sequence will be smaller than amino-or carboxyl-terminal fusions and of the order of 1-4 amino acid residues.

TABLE 1

Amino Acid	Three-letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any amino acid as above	Xaa	X

TABLE 2

Non-conventional amino acid	Code	Non-conventional amino acid	Code
α-aminobutyric acid	Abu	L-N-methylalanine	Nmala
α-amino-α-methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane-carboxylate	Cpro	L-N-methylasparagine	Nmasn
		L-N-methylaspartic acid	Nmasp
aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl-carboxylate	Norb	L-N-methylglutamine	Nmgln
		L-N-methylglutamic acid	Nmglu
cyclohexylalanine	Chexa	L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisolleucine	Nmile
D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
D-threonine	Dthr	L-norleucine	Nle
D-tryptophan	Dtrp	L-norvaline	Nva

D-tyrosine	Dtyr	α-methyl-aminoisobutyrate	Maib
D-valine	Dval	α-methyl-γ-aminobutyrate	Mgabu
D-α-methylalanine	Dmala	α-methylcyclohexylalanine	Mchexa
D-α-methylarginine	Dmarg	α-methylcylcopentylalanine	Mcpen
D-α-methylasparagine	Dmasn	α-methyl-α-napthylalanine	Manap
D-α-methylaspartate	Dmasp	α-methylpenicillamine	Mpen
D-α-methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
D-α-methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
D-α-methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
D-α-methylisoleucine	Dmile	N-amino-α-methylbutyrate	Nmaabu
D-α-methylleucine	Dmleu	α-napthylalanine	Anap
D-α-methyllysine	Dmlys	N-benzylglycine	Nphe
D-α-methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
D-α-methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
D-α-methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
D-α-methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
D-α-methylserine	Dmser	N-cyclobutylglycine	Ncbut
D-α-methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
D-α-methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
D-α-methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
D-α-methylvaline	Dmval	N-cylcododecylglycine	Ncdod
D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl) glycine	Nbhm
D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl) glycine	Nbhe

D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl) glycine	Narg
D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
D-N-methylhistidine	Dnmhis	N-(hydroxyethyl))glycine	Nser
D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)) glycine	Nhis
D-N-methylleucine	Dnmleu	N-(3-indolylyethyl) glycine	Nhtrp
D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
D-N-methyltyrosine	Dnmtyr	N-methyla-napthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
γ-aminobutyric acid	Gabu	N-(*p*-hydroxyphenyl)glycine	Nhtyr
L-*t*-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L-α-methylalanine	Mala
L-α-methylarginine	Marg	L-α-methylasparagine	Masn
L-α-methylaspartate	Masp	L-α-methyl-*t*-butylglycine	Mtbug
L-α-methylcysteine	Mcys	L-methylethylglycine	Metg
L-α-methylglutamine	Mgln	L-α-methylglutamate	Mglu
L-α-methylhistidine	Mhis	L-α-methylhomo phenylalanine	Mhphe
L-α-methylisoleucine	Mile	N-(2-methylthioethyl) glycine	Nmet
L-α-methylleucine	Mleu	L-α-methyllysine	Mlys

L-α-methylmethionine	Mmet	L-α-methylnorleucine	Mnle
L-α-methylnorvaline	Mnva	L-α-methylornithine	Morn
L-α-methylphenylalanine	Mphe	L-α-methylproline	Mpro
L-α-methylserine	Mser	L-α-methylthreonine	Mthr
L-α-methyltryptophan	Mtrp	L-α-methyltyrosine	Mtyr
L-α-methylvaline	Mval	L-N-methylhomo phenylalanine	Nmhphe
N-(N-(2,2-diphenylethyl) carbamylmethyl)glycine	Nnbhm	N-(N-(3,3-diphenylpropyl) carbamylmethyl)glycine	Nnbhe
1-carboxy-1-(2,2-diphenyl-ethylamino)cyclopropane	Nmbc		

The present invention provides for a method of treatment or prophylaxis of disease conditions associated with neuronal death or where cell death is to be promoted such as in treating or preventing cancer growth and/or development.

In one embodiment, it has been determined in accordance with the present invention that expression of a nucleic acid molecule encoding only death signal and not adjacent, proximal or juxtaposed to a membrane-associating sequence results in antagonising of the death signal.

According to this embodiment, the present invention contemplates a method for inhibiting, reducing or otherwise antagonising a $p75^{NTR}$-mediated death signal in a neuronal cell, said method comprising introducing a nucleic acid molecule capable of being expressed to an expression product which corresponds to a non-membrane associated form of the $p75^{NTR}$ death signal region or a derivative, functional equivalent or homologue thereof.

In a related embodiment there is provided a method for inhibiting, reducing or otherwise antagonising a $p75^{NTR}$-mediated death signal in a neuronal cell, said method comprising contacting a cell carrying a $p75^{NTR}$ with a death signal-inhibiting effective amount of a molecule capable of antagonising the death signal of $p75^{NTR}$ or a component of the death

signalling pathway.

This aspect of the present invention is useful for the treatment of a range of neurodegenerative diseases such as cerebral palsy, trauma induced paralysis, vascular ischaemia associated with stroke, neuronal tumours, motoneurone disease, Parkinson's disease, Huntington's disease, Alzheimer's disease, multiple sclerosis and peripheral neuropathies associated with diabetes, heavy metal or alcohol toxicity, renal failure and/or infectious diseases such as herpes, rubella, measles, chicken pox, HIV and/or HTLV-1. This aspect is also useful for treating neurons damaged by trauma or disease.

Alternatively, the method is aimed at targetting certain cells such as cancer cells wherein expression is required of a death signal from $p75^{NTR}$ or a derivative, functional equivalent or homologue thereof adjacent, proximal or otherwise juxtaposed to a membrane-associating portion of $p75^{NTR}$ or other membrane molecules or is in multimeric form. The nucleic acid molecule may require modification to ensure appropriate targetting to the cell or the nucleic acid molecule may be injected directly into cancerous tissue.

Another aspect of the present invention provides a biological composition comprising a genetic molecule capable of being expressed into a $p75^{NTR}$ death signal antagonist or a $p75^{NTR}$ death signal. The biological composition further comprises one or more pharmaceutically acceptable carriers and/or diluents. The nucleic acid molecules according to this aspect of the present invention may be naked nucleic acid molecules or contained or associated with a viral vector or other suitable delivery mechanism.

Another aspect of the present invention is directed to a biological composition comprising a molecule capable of antagonising $p75^{NTR}$-mediated death signalling of a cell.

Suitable molecules according to this aspect of the present invention are as contemplated above and include a peptide, polypeptide or protein comprising a soluble form of the $p75^{NTR}$ death signalling region or an antagonist of a component of the $p75^{NTR}$ death signalling pathway.

The present invention is also useful as a culture agent such as preventing or reducing the death of cells *in vitro*. The present invention is particularly useful *in vitro* when used in combination with LIF. Even more particularly, the present invention is useful for culturing recombinant cell lines.

The present invention also provides for the use of the death signal of $p75^{NTR}$ in the manufacture of a medicament for the treatment of neurodegenerative diseases in animals. Preferred animals include humans, primates, livestock animals, laboratory test animals, companion animals and captive wild animals.

The present invention is further described by the following non-limiting Examples.

EXAMPLE 1

The aim of this example was to determine the protein domains on $p75^{NTR}$ responsible for death signalling.

In order to investigate how $p75^{NTR}$ signals neuronal death, the inventors devised a robust *in vitro* assay for $p75^{NTR}$ induced death. Plasmid expression constructs were microinjected into individual neurons in the presence of the growth factor LIF, and the survival of the neurons expressing the different plasmids was determined. A series of plasmid constructs which encode incomplete $p75^{NTR}$ proteins were made (see Figure 1) and the ability of each protein to signal death when over expressed was assessed.

The $p75^{NTR}$ protein is a transmembrane protein comprised of a large extracellular domain with four cysteine rich motifs responsible for interacting with soluble growth factors, and a short cytoplasmic, intracellular tail. The cytoplasmic domain does not contain a kinase domain but contains a domain with significant homology to a motif known as a "death domain", found in apoptosis-inducing Tumour Necrosis Factor Receptors (TNFR) and TNFR-associating death-effector proteins [9].

Using expression plasmids of p75^{NTR} proteins deleted for either the entire cytoplasmic domain (p75nc) or a significant portion of the cytoplasmic domain including the entire death domain (p75tm), the inventors found that the cytoplasmic domain is responsible for death signalling. Surprisingly, the intracellular 35 amino acid domain juxtaposed to the membrane, and not the death domain, is responsible for death signalling (Figure 2). This region of the p75^{NTR} protein shows no homology to other death inducing proteins or to known functional motifs.

To further investigate the domain required for death signalling the inventors made constructs expressing p75^{NTR} proteins deleted for the extracellular domain or the extracellular and transmembrane domains. Proteins without extracellular domains retain the signal peptide which is responsible for correctly transporting the protein into the cell membrane. Proteins without transmembrane domains are expressed free in the cytoplasm of the cell and are epitope tagged with a FLAG motif for detection.

The inventors found that the extracellular domain of p75^{NTR} had a significant inhibitory effect of the ability of the cytoplasmic domain to signal cell death. Furthermore, the membrane linked 35 amino acid cytoplasmic domain (c35) was a potent stimulant of neuronal death with over 90% of cells injected with the plasmid dead after 16 hours (Figure 3). However, if the cytoplasmic 35 amino acid domain is not associated with the membrane, the ability of the domain to induce death is removed (Figure 4).

These results indicate that the domain responsible for death induction is within the first 35 amino acids of the cytoplasmic tail but that the transmembrane domain, or at least association with the membrane, is required for death-signal activation. This may be related to the ability of the transmembrane protein to more efficiently form death-signal inducing multimers, or that the position of the p75^{NTR} protein in relation to other membrane-bound accessory molecules might be important in initiating death signalling.

The inventors hypothesised that the free cytoplasmic expressed 35 amino acid domain might be able to interfere with death signalling from full length p75^{NTR} proteins by a dominant-negative mechanism, and attempted to inhibit the death by co-expressing the proteins. Given

the results presented below regarding the ability of overexpression of Bcl-2 to enhance $p75^{NTR}$ killing, this paradigm was used to test the ability of the c35 protein to inhibit death signalling. The inventors found that indeed the expression of the c35 protein was able to inhibit this killing (Figure 5). This further indicates that $p75^{NTR}$ signals killing *via* interaction of an accessory molecule to a motif within the first 35 amino acids of the cytoplasmic domain.

EXAMPLE 2

The aim of this example is to determine the minimum number of amino acid residues on c35 require to mediate death signalling.

A series of deletion and truncation mutants in the c35 region are produced and tested for the ability to induce death signalling.

The deletion mutants from the membrane distal end are as follows:

KRWNSCKQNKQGANSRPVNQTPPPEGEKLHSDSG;
KRWNSCKQNKQGANSRPVNQTPPPEGEKLHSDS;
KRWNSCKQNKQGANSRPVNQTPPPEGEKLHSD;
KRWNSCKQNKQGANSRPVNQTPPPEGEKLHS;
KRWNSCKQNKQGANSRPVNQTPPPEGEKLH;
KRWNSCKQNKQGANSRPVNQTPPPEGEKL;
KRWNSCKQNKQGANSRPVNQTPPPEGEK;
KRWNSCKQNKQGANSRPVNQTPPPEGE;
KRWNSCKQNKQGANSRPVNQTPPPEG;
KRWNSCKQNKQGANSRPVNQTPPPE;
KRWNSCKQNKQGANSRPVNQTPPP;
KRWNSCKQNKQGANSRPVNQTPP;
KRWNSCKQNKQGANSRPVNQTP;
KRWNSCKQNKQGANSRPVNQT;
KRWNSCKQNKQGANSRPVNQ;

KRWNSCKQNKQGANSRPVN;
KRWNSCKQNKQGANSRPV;
KRWNSCKQNKQGANSRP;
KRWNSCKQNKQGANSR;
KRWNSCKQNKQGANS;
KRWNSCKQNKQGAN;
KRWNSCKQNKQGA;
KRWNSCKQNKQG;
KRWNSCKQNKQ;
KRWNSCKQNK;
KRWNSCKQN;
KRWNSCKQ;
KRWNSCK;
KRWNSC;
KRWNS;
KRWN;
KRW;
KR; and
K.

The deletion mutants from the membrane proximal end are as follows:

RWNSCKQNKQGANSRPVNQTPPPEGEKLHSDSGI;
WNSCKQNKQGANSRPVNQTPPPEGEKLHSDSGI;
NSCKQNKQGANSRPVNQTPPPEGEKLHSDSGI;
SCKQNKQGANSRPVNQTPPPEGEKLHSDSGI;
CKQNKQGANSRPVNQTPPPEGEKLHSDSGI;
KQNKQGANSRPVNQTPPPEGEKLHSDSGI;
QNKQGANSRPVNQTPPPEGEKLHSDSGI;
NKQGANSRPVNQTPPPEGEKLHSDSGI;
KQGANSRPVNQTPPPEGEKLHSDSGI;

QGANSRPVNQTPPPEGEKLHSDSGI;
GANSRPVNQTPPPEGEKLHSDSGI;
ANSRPVNQTPPPEGEKLHSDSGI;
NSRPVNQTPPPEGEKLHSDSGI;
SRPVNQTPPPEGEKLHSDSGI;
RPVNQTPPPEGEKLHSDSGI;
PVNQTPPPEGEKLHSDSGI;
VNQTPPPEGEKLHSDSGI;
NQTPPPEGEKLHSDSGI;
QTPPPEGEKLHSDSGI;
TPPPEGEKLHSDSGI;
PPPEGEKLHSDSGI;
PPEGEKLHSDSGI;
PEGEKLHSDSGI;
EGEKLHSDSGI;
GEKLHSDSGI;
EKLHSDSGI;
KLHSDSGI;
LHSDSGI;
HSDSGI;
SDSGI;
DSGI;
SGI;
GI; and
I.

EXAMPLE 3

ROLE OF BCL-2 IN PROMOTING p75^{NTR} MEDIATED DEATH SIGNALLING

As the inventors had shown that the death of dorsal root ganglia (DRG) sensory neurons *in vitro* and *in vivo*, was, at least in part, mediated by p75^{NTR}, p75^{NTR} was over-expressed in these cells by microinjecting rat p75^{NTR} cDNA expressing plasmid into the nucleus of mouse sensory neurons. These were cultured in the presence of the LIF to prevent neuronal death not linked to p75^{NTR} mechanisms. It was found that the expression of the rat p75^{NTR} could be detected by surface immunofluorescence within 24 hours of injection. The injected neurons were observed over a 48 hour period and the viability was assessed. It was found that within the first 16 hours, a significantly higher number of p75^{NTR} plasmid injected neurons had died compared to neurons injected with control plasmids β-galactosidase, or a truncated p75^{NTR} protein lacking the entire cytoplasmic domain (p75^{NTR}nc). It was found that p75^{NTR}-mediated neuronal death occurred later in the experiment similar to Fas/TNF-induced rapid cell death. Since both full-length p75^{NTR} and p75^{NTR}nc protein showed a similar level of expression after injection, this indicates that the cytoplasmic domain of p75^{NTR} is required for death signalling. This was expected since the cytoplasmic tail contains a sequence with homology to the Fas/TNFR "death domain" [9].

The inventors next examined whether deletion of the "death domain" also abolished the ability of p75^{NTR} to kill. It was found that the neuronal death observed after expression of p75^{NTR} with a truncated cytoplasmic tail (p75^{NTR}tr) was equivalent to the full-length p75^{NTR} protein. This demonstrated that the "death domain" was not required for p75^{NTR} killing and, since the p75^{NTR} death domain has recently been shown to have a different tertiary structure to TNFR family death domain and does not self-associate *in vitro*, it suggests that the p75^{NTR} "death domain" may not normally function to induce death. Together, these results predict that an alternative pathway involving proteins other than "death domain" adapter proteins, such as TRADD and FADD, is responsible for p75^{NTR}-mediated killing.

The Bcl-2 family of proteins is involved in mediating apoptotic signalling pathways, and can homodimerise or heterodimerise with other family members. Bcl-2 and Bcl-xL are well characterised inhibitors of stress-induced apoptosis, JNK activation and neuronal death due to growth-factor limitation. However, both are poor inhibitors of Fas and TNFR mediated opoptosis. As it had been shown previously that high levels of Bcl-2 or Bcl-xL blocked neuronal cell death in a variety of models, the inventors examined whether over-expression of these proteins could block the death induced by $p75^{NTR}$.

The inventors found that over-expression of Bcl-xL protected neurons against $p75^{NTR}$-induced death, supporting the hypothesis that $p75^{NTR}$ signals through an alternative pathway to TNFR-induced apoptosis. In contrast, while Bcl-2 over-expression alone had no effect on cell survival in the presence of LIF, Bcl-2 in combination with $p75^{NTR}$ over-expression, surprisingly, induced a significant increase in neuronal death above that seen with $p75^{NTR}$ over-expression alone. Bcl-2 in combination with $p75^{NTR}$nc did not cause significant cell death and furthemore, the cell death observed with $p75^{NTR}$ and Bcl-2 over-expression was totally ablated if the cells were cultured in NGF. Bcl-2 was able to protect against neuronal death induced by NGF withdrawal, but not withdrawal of LIF. Thus, at the same expression levels in the same neuronal population, Bcl-2 was able to prevent or enhance neuronal cell death depending on the nature of the death signal.

These results are surprising since Bcl-2 has previously been shown to have similar actions to Bcl-xL in almost all cell-death systems.

To determine whether the paradoxical effect of Bcl-2 on $p75^{NTR}$-induced killing was related to its kown anti-apoptotic activity, Bcl-2 proteins with inactivating point mutation, G145E, in the "Bcl-2 Homology" BH1 domain and W188A in the BH2 domain were utilised. Like wildtype Bcl-2, expression of either Bcl-2 mutant alone did not effect neuronal survival. In combination with $p75^{NTR}$ expression, the enhanced killing effect seen with Bcl-2 co-expression was abrogated by the G145E mutation, even though the proteins were expressed to comparable levels. Thus, an intact BH1 homology region is required for the death promoting activity of Bcl-2.

Mutation of the equivalent G138 residue in Bcl-xL results in a conformational change between α-helices 4 and 5, disrupting access to the hydrophobic cleft formed by BH1, BH2 and BH3 domains. Therefore, the molecular mechanism by which Bcl-2 participates in the $p75^{NTR}$ killing pathway may be dependent on interactions either directly with the BH domains or with the hydrophobic cleft, as indicated with experiments using the W188A mutation. Co-expression of $p75^{NTR}$ with the Bcl-2 W188A protein not only abrogated the increased $p75^{NTR}$ killing but, more importantly, protected neurons from any $p75^{NTR}$-induced death, reminiscent of that seen with Bcl-xL. These experiments suggest that the conformation of the Bcl-2 protein is integral to the opposing functions observed herein.

The inventors had observed that DRG neurons isolated from newborn mice depleted for $p75^{NTR}$ were less susceptible to NGF withdrawal, as is the case with sympathetic neurons, when compared to neurons from wildtype mice. This is indicative of absent or delayed naturally occurring cell death observed in these mice. The inventors attempted to induce cell death in $p75^{NTR}$ "knock out" DRG neurons by re-introducing $p75^{NTR}$ expression. Surprisingly, apoptosis was not induced by re-expression into "knock out" DRG neurons, the inventors found that neuronal death was significantly increased under these conditions. This implicated an absolute requirement for Bcl-2 in mediating $p75^{NTR}$ killing.

The inventors tested, therefore, whether high endogenous Bcl-2 levels might be necessary for successful $p75^{NTR}$-mediated killing in normal neurons by assaying $p75^{NTR}$ killing in Bcl-2 depleted cells. Endogenous Bcl-2 was down regulated by antisense as previously described. When the Bcl-2 antisense plasmid was injected at the same time as $p75^{NTR}$ plasmids no diminishment in the death signal was seen. If, however, the Bcl-2 antisense was microinjected first (to give time to reduced Bcl-2 production and deplete endogenous Bcl-2; and then a day later the $p75^{NTR}$ or $p75^{NTR}$nc constructs were microinjected, there was no difference in survival between $p75^{NTR}$ and $p75^{NTR}$nc expressing neurons, strongly suggesting that endogenous Bcl-2 is required for $p75^{NTR}$ killing effects. To confirm this observation, the inventors isolated neurons from newborn Bcl-2 "knock out" mice (an heterozygous line of mice containing a disrupted Bcl-2 gene) and their wild-type litter mates and compared the effect of $p75^{NTR}$ over-expression with control plasmid $p75^{NTR}$. It was found that the neurons

isolated from Bcl-2 deficient mice were significantly protected from $p75^{NTR}$ killing, showing a 56.9% (n=3) reduction in death compared wildtype neurons, supporting the hypothesis that endogenous Bcl-2 is required for $p75^{NTR}$ killing.

Bcl-2 has previously been observed to increase cell death when highly expressed both *in vitro* and *in vivo* when expressed at high levels as a transgene, causing increased apoptosis in the brain under a neuron specific promoter, or in photoreceptor cells when expressed specifically under a rhodopsin promoter. Thus, it is possible that the high level of Bcl-2 is able to "prime" the death pathway such that an apoptotic stimulus *via* $p75^{NTR}$ results in rapid cell death.

Bcl-2 and Bcl-xL when cleaved by caspases have also been shown to be capable of promoting apoptosis *in vitro*, with cells expressing non-cleavable mutant Bcl-2 and Bcl-xL proteins showing increased viability compared to cells expressing wildtype proteins. Cleavage of Bcl-2 is possible in this sytem, however, the Bcl-2 mutations which results in loss of death promoting activity, would not prevent cleavage of Bcl-2, indicating that cleavage of Bcl-2 would only be part of the mechanism by which Bcl-2 promotes killing. In addition, if cleavage was the dominant mechanism, Bcl-xL might be expected to act as a death signalling protein in this system.

To investigate whether the $p75^{NTR}$-Bcl-2 death-signalling cascade was dependent on caspase activation, inhibitors of caspases were employed. In the presence of z-VAD, a nonspecific caspase peptide inhibitor, or after co-expression of modified crmA plasmids, designed to inhibit Group II caspases such as caspases 2 and 3, $p75^{NTR}$-mediated death was significantly reduced. Similarly, the modified crmA was able to block the killing induced by co-expression of $p75^{NTR}$ and Bcl-2. This indicates that $p75^{NTR}$ induced apoptosis is a caspase dependent pathway and that the mechanism by which Bcl-2 assists killing is through the same pathway.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

BIBLIOGRAPHY

1. Levi-Montalcini, 1982. Ann. Rev. Neurosci. 5: 341-362.
2. Rabizadeh,et al., Science, 1993. 261(5119): p. 345-8.
3. Majdan, et al., Journal of Neuroscience, 1997. 17(18): p. 6988-98.
4. Barrett, G.L. and A. Georgiou, Journal of Neuroscience Research, 1996. 45(2): p. 117-28.
5. Barrett, G.L. and P.F. Bartlett, Proceedings of the National Academy of Sciences of the United States of America, 1994. 91(14): p. 6501-5.
6. Cheema, S.S., G.L. Barrett, and P.F. Bartlett, Journal of Neuroscience Research, 1996. 46(2): p. 239-45.
7. Bamji, S.X., et al., Journal of Cell Biology, 1998. 140(4): p. 911-23.
8. Van der Zee, et al., Science, 1996. 274(5293): p. 1729-32.
9. Feinstein, et al.,Trends in Biochemical Sciences, 1995. 20(9): p. 342-4.
10. Moix, et al., Brain Research, 1991. 564(1): p. 176-80.
11. Lee, et al., Journal of Neuroscience Research, 1995. 41(5): p. 684-95.
12. Rende, et al., Journal of Comparative Neurology, 1995. 363(2): p. 249-63.
13. Seeburger, et al., Brain Research, 1993. 621(1): p. 111-5.
14. De Simone, et al., Neuropathology & Applied Neurobiology, 1996. 22(1): p. 54-9.
15. Conner, et al., Journal of Comparative Neurology, 1992. 319(3): p. 454-62.
16. Wiley, et al., Journal of the Neurological Sciences, 1995. 128(2): p. 157-66.
17. Needleman and Wunsch, 1970. J. Mol. Biol. 48: 443-453.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH

(ii) TITLE OF INVENTION: A METHOD OF MODULATING CELL SURVIVAL AND REAGENTS USEFUL FOR SAME-II

(iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: DAVIES COLLISON CAVE
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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
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(B) FILING DATE: 07-OCT-1998

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: AU PROVISIONAL
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3260 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..3260

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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ACA GCT CCG GCG GGC AGC AGG CGC TGG AGC GCA TCG CAG TTC AGC TCA        48
Thr Ala Pro Ala Gly Ser Arg Arg Trp Ser Ala Ser Gln Phe Ser Ser
  1               5                  10                  15

GCG CAG CAC CAT CGG TCT GCG GAG CGG ACT GAG CTA GAA GCG GAG CGC        96
Ala Gln His His Arg Ser Ala Glu Arg Thr Glu Leu Glu Ala Glu Arg
             20                  25                  30

TGA CGC CGG AGG CGT GCA ATG AGG AGG GCA GGT GCT GCC TGC AGC GCC       144
 *  Arg Arg Arg Arg Ala Met Arg Arg Ala Gly Ala Ala Cys Ser Ala
         35                  40                  45

ATG GAC CGG CTG CGC CTG CTG CTG CTG CTG ATT CTA GGG GTG TCC TCT       192
Met Asp Arg Leu Arg Leu Leu Leu Leu Leu Ile Leu Gly Val Ser Ser
     50                  55                  60

GGA GGT GCC AAG GAG ACA TGT TCC ACA GGC CTG TAC ACC CAC AGC GGA       240
Gly Gly Ala Lys Glu Thr Cys Ser Thr Gly Leu Tyr Thr His Ser Gly
 65                  70                  75                  80

GAG TGC TGC AAA GCC TGC AAC TTG GGC GAA GGC GTG GCC CAG CCC TGC       288
Glu Cys Cys Lys Ala Cys Asn Leu Gly Glu Gly Val Ala Gln Pro Cys
                 85                  90                  95

GGA GCC AAC CAG ACC GTG TGT GAA CCC TGC CTG GAC AAT GTT ACA TTC       336
Gly Ala Asn Gln Thr Val Cys Glu Pro Cys Leu Asp Asn Val Thr Phe
            100                 105                 110

TCC GAT GTG GTG AGC GCC ACT GAG CCG TGC AAG CCG TGC ACC GAG TGC       384
Ser Asp Val Val Ser Ala Thr Glu Pro Cys Lys Pro Cys Thr Glu Cys
        115                 120                 125

CTG GGC CTG CAG AGC ATG TCC GCT CCC TGT GTG GAG GCA GAC GAT GCA       432
Leu Gly Leu Gln Ser Met Ser Ala Pro Cys Val Glu Ala Asp Asp Ala
    130                 135                 140

GTG TGC AGA TGT GCC TAT GGC TAC TAC CAG GAC GAG GAG ACT GGC CAC       480
Val Cys Arg Cys Ala Tyr Gly Tyr Tyr Gln Asp Glu Glu Thr Gly His
145                 150                 155                 160

TGT GAG GCT TGC AGC GTG TGC GAG GTG GGC TCG GGA CTC GTG TTC TCC       528
Cys Glu Ala Cys Ser Val Cys Glu Val Gly Ser Gly Leu Val Phe Ser
                165                 170                 175

TGC CAG GAC AAA CAG AAC ACA GTG TGT GAA GAG TGC CCA GAG GGC ACA       576
Cys Gln Asp Lys Gln Asn Thr Val Cys Glu Glu Cys Pro Glu Gly Thr
            180                 185                 190
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TAC TCA GAC GAA GCC AAC CAC GTG GAC CCG TGC CTA CCC TGC ACG GTG      624
Tyr Ser Asp Glu Ala Asn His Val Asp Pro Cys Leu Pro Cys Thr Val
        195                 200                 205

TGC GAG GAC ACT GAG CGC CAG TTA CGC GAG TGC ACG CCC TGG GCT GAT      672
Cys Glu Asp Thr Glu Arg Gln Leu Arg Glu Cys Thr Pro Trp Ala Asp
    210                 215                 220

GCT GAA TGC GAA GAG ATC CCT GGT CGA TGG ATC CCA AGG TCT ACG CCC      720
Ala Glu Cys Glu Glu Ile Pro Gly Arg Trp Ile Pro Arg Ser Thr Pro
225                 230                 235                 240

CCG GAG GGC TCC GAC AGC ACA GCG CCC AGC ACC CAG GAG CCT GAG GTT      768
Pro Glu Gly Ser Asp Ser Thr Ala Pro Ser Thr Gln Glu Pro Glu Val
                245                 250                 255

CCT CCA GAG CAA GAC CTT GTA CCC AGT ACA GTG GCG GAT ATG GTG ACC      816
Pro Pro Glu Gln Asp Leu Val Pro Ser Thr Val Ala Asp Met Val Thr
            260                 265                 270

ACT GTG ATG GGC AGC TCC CAG CCT GTA GTG ACC CGC GGC ACC ACC GAC      864
Thr Val Met Gly Ser Ser Gln Pro Val Val Thr Arg Gly Thr Thr Asp
        275                 280                 285

AAC CTC ATT CCT GTC TAT TGC TCC ATC TTG GCT GCT GTG GTC GTG GGC      912
Asn Leu Ile Pro Val Tyr Cys Ser Ile Leu Ala Ala Val Val Val Gly
    290                 295                 300

CTT GTG GCC TAT ATT GCT TTC AAG AGG TGG AAC AGC TGC AAA CAA AAT      960
Leu Val Ala Tyr Ile Ala Phe Lys Arg Trp Asn Ser Cys Lys Gln Asn
305                 310                 315                 320

AAA CAA GGC GCC AAC AGC CGC CCC GTG AAC CAG ACG CCC CCA CCG GAG     1008
Lys Gln Gly Ala Asn Ser Arg Pro Val Asn Gln Thr Pro Pro Pro Glu
                325                 330                 335

GGA GAG AAA CTG CAC AGC GAC AGT GGC ATC TCT GTG GAC AGC CAG AGC     1056
Gly Glu Lys Leu His Ser Asp Ser Gly Ile Ser Val Asp Ser Gln Ser
            340                 345                 350

CTG CAC GAC CAG CAG ACC CAT ACG CAG ACT GCC TCA GGC CAG GCC CTC     1104
Leu His Asp Gln Gln Thr His Thr Gln Thr Ala Ser Gly Gln Ala Leu
        355                 360                 365

AAG GGT GAT GGC AAC CTC TAC AGT AGC CTG CCC CTG ACC AAG CGT GAG     1152
Lys Gly Asp Gly Asn Leu Tyr Ser Ser Leu Pro Leu Thr Lys Arg Glu
    370                 375                 380

GAG GTA GAG AAA CTG CTC AAC GGG GAT ACC TGG CGA CAT CTG GCA GGC     1200
Glu Val Glu Lys Leu Leu Asn Gly Asp Thr Trp Arg His Leu Ala Gly
385                 390                 395                 400

GAG CTG GGT TAC CAG CCT GAA CAT ATA GAC TCC TTT ACC CAC GAG GCC     1248
Glu Leu Gly Tyr Gln Pro Glu His Ile Asp Ser Phe Thr His Glu Ala
                405                 410                 415

TGC CCA GTG CGA GCC CTG CTG GCC AGC TGG GGT GCC CAG GAC AGT GCA     1296
Cys Pro Val Arg Ala Leu Leu Ala Ser Trp Gly Ala Gln Asp Ser Ala
            420                 425                 430

ACG CTT GAT GCC CTT TTA GCC GCC CTG CGA CGC ATC CAG AGA GCT GAC     1344
Thr Leu Asp Ala Leu Leu Ala Ala Leu Arg Arg Ile Gln Arg Ala Asp
        435                 440                 445

ATT GTG GAG AGT CTA TGC AGC GAG TCC ACT GCC ACA TCC CCA GTG TGA     1392
Ile Val Glu Ser Leu Cys Ser Glu Ser Thr Ala Thr Ser Pro Val  *
    450                 455                 460
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ACT CAC AGA CTG GGA GCC CCT GTC CTG TCC CAC ATT CCG ACG ACT GAT      1440
Thr His Arg Leu Gly Ala Pro Val Leu Ser His Ile Pro Thr Thr Asp
465                 470                 475                 480

GTT CTA GCC AGC CCC CAC AGA GCT GCC CCC TCT CCC TCG GGG ATG GCC      1488
Val Leu Ala Ser Pro His Arg Ala Ala Pro Ser Pro Ser Gly Met Ala
            485                 490                 495

CAA CGG TCA GAA CGG AGC ATC TCT GTG CAG GGC CTC TGT GTT CCC ACT      1536
Gln Arg Ser Glu Arg Ser Ile Ser Val Gln Gly Leu Cys Val Pro Thr
        500                 505                 510

CCT GAC TCC GTT GCT GCT CCC GAG GGG GCC CTT GCT TCT GAC CAC CCT      1584
Pro Asp Ser Val Ala Ala Pro Glu Gly Ala Leu Ala Ser Asp His Pro
    515                 520                 525

CTC CTC AGC AAG AGA GAG AGA GAG GAC CAC CCG AGC CTG ACT TGC TCC      1632
Leu Leu Ser Lys Arg Glu Arg Glu Asp His Pro Ser Leu Thr Cys Ser
530                 535                 540

ATT TCC ATC TCA GGC CTT TCC TTC CTT TCT ACA CAT TAG CTG TGT CAG      1680
Ile Ser Ile Ser Gly Leu Ser Phe Leu Ser Thr His  *  Leu Cys Gln
545                 550                 555                 560

ATC TGG GGG TTT GAC ACT AGG AGA AGG GAG CGG GGG CAC CCC TAA GAC      1728
Ile Trp Gly Phe Asp Thr Arg Arg Arg Glu Arg Gly His Pro  *  Asp
            565                 570                 575

TCA GGA GGT ACT GAA GAA CCA GAG CCA TGG ACT CCA CAC TGT GAA CCG      1776
Ser Gly Gly Thr Glu Glu Pro Glu Pro Trp Thr Pro His Cys Glu Pro
        580                 585                 590

GAG AAC AAG GGG CGG GGC ATT GTG GTA GGC TAG ACC TTC CTT AGC CCC      1824
Glu Asn Lys Gly Arg Gly Ile Val Val Gly  *  Thr Phe Leu Ser Pro
    595                 600                 605

TCC CTT CTC CCC TCT GGC CAA AGA AGA GGA TTA CGG ACC TAT CTG AGC      1872
Ser Leu Leu Pro Ser Gly Gln Arg Arg Gly Leu Arg Thr Tyr Leu Ser
610                 615                 620

TGA AAG CAG GTT TGG AAC CCA GCC CAC ACT TCT CTC TCA CAC ACA GGA      1920
 *  Lys Gln Val Trp Asn Pro Ala His Thr Ser Leu Ser His Thr Gly
625                 630                 635                 640

TGG TAA AAC CCA GAG AAA GGC AGG GAC TGA CCT AGG CCA CCC AAC CAC      1968
Trp  *  Asn Pro Glu Lys Gly Arg Asp  *  Pro Arg Pro Pro Asn His
            645                 650                 655

AGG AAG AAC AAA TGA AGG CTG ATA CAC TCC GTT TCT GAA TGA GGG CGT      2016
Arg Lys Asn Lys  *  Arg Leu Ile His Ser Val Ser Glu  *  Gly Arg
        660                 665                 670

CAA GTG TGC TTG TTG ACA GGG ATG GCG TGA CTT TCA GGG AAA TAT CTG      2064
Gln Val Cys Leu Leu Thr Gly Met Ala  *  Leu Ser Gly Lys Tyr Leu
    675                 680                 685

GAA GCC ATG TCT GCC CCG CCC TCA ACC ACT TCC AGG CCC CTA CCC AAC      2112
Glu Ala Met Ser Ala Pro Pro Ser Thr Thr Ser Arg Pro Leu Pro Asn
690                 695                 700

CCT TGT GCA GAT GAA CTG TTT GTT CAA GGG CTG GTC CAT TGG TCT ATT      2160
Pro Cys Ala Asp Glu Leu Phe Val Gln Gly Leu Val His Trp Ser Ile
705                 710                 715                 720

CTG ATG GAG TCA AGC TAA GGG CTC AGG CTT ATC CAT AAG GCA TTT GTG      2208
Leu Met Glu Ser Ser  *  Gly Leu Arg Leu Ile His Lys Ala Phe Val
            725                 730                 735
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GAG AGA TGA ATC TGT TAG TGC GCT CAT TCT TGG CAT AAG CCT GAA GCC 2256
Glu Arg * Ile Cys * Cys Ala His Ser Trp His Lys Pro Glu Ala
740 745 750

AAC ACG GCC CTT AAT GTC AGC CCT CGG GGT CAG GAA CCA AGG ACT CCC 2304
Asn Thr Ala Leu Asn Val Ser Pro Arg Gly Gln Glu Pro Arg Thr Pro
755 760 765

ACC CCA CAA TCC AAC ACT ATA CTA CAT TAC ACA CAC ACA CAC ACA CAC 2352
Thr Pro Gln Ser Asn Thr Ile Leu His Tyr Thr His Thr His Thr His
770 775 780

ACA CAC ACA CAC ACA CAC ACA CAC ACA GAT ATC TTG CTT TTC TCC CCA 2400
Thr His Thr His Thr His Thr His Thr Asp Ile Leu Leu Phe Ser Pro
785 790 795 800

TGG CTC TTT TGG GGC TGA GAC TAG ATC CTG CTG GGA GTC ACT GCC AGT 2448
Trp Leu Phe Trp Gly * Asp * Ile Leu Leu Gly Val Thr Ala Ser
805 810 815

GAG AGA TCC GGA GGG GAC AGA GCT GAG CTT CAT GGG GCT GTC TTC CTC 2496
Glu Arg Ser Gly Gly Asp Arg Ala Glu Leu His Gly Ala Val Phe Leu
820 825 830

GCC CCC GGG TCT GGC AGG CCA AGA ATG ACT GCA TCT GAG CTG GTG TCT 2544
Ala Pro Gly Ser Gly Arg Pro Arg Met Thr Ala Ser Glu Leu Val Ser
835 840 845

GTC TTC CAA TGG CCT GTG CGT GGA GGA AAT GCT CCC ACT CCT CCC CTT 2592
Val Phe Gln Trp Pro Val Arg Gly Gly Asn Ala Pro Thr Pro Pro Leu
850 855 860

CTT GAA GCT GCC CCC AGA AGA CTA CAG TGC AAA AGA GCA GAC TGG TGT 2640
Leu Glu Ala Ala Pro Arg Arg Leu Gln Cys Lys Arg Ala Asp Trp Cys
865 870 875 880

GAG AAC ACA AGA AAA AGC AGA TGC TGG CCC TGC AGT CTG TGG CAG CTT 2688
Glu Asn Thr Arg Lys Ser Arg Cys Trp Pro Cys Ser Leu Trp Gln Leu
885 890 895

TCT CCT CAG CTT CAA GGC CCC TGC AAA GGA CGG ATT TCC TGA GCA CGG 2736
Ser Pro Gln Leu Gln Gly Pro Cys Lys Gly Arg Ile Ser * Ala Arg
900 905 910

CCA GGA AGG GGC AAG AGG GTT CGG TTC AGT GGC GCT TTC TCC CGG CTC 2784
Pro Gly Arg Gly Lys Arg Val Arg Phe Ser Gly Ala Phe Ser Arg Leu
915 920 925

CTT GGC CTG TTC TGT TTT GCT TGC TGT TGG AAT GAG TGG GCA CCC CCT 2832
Leu Gly Leu Phe Cys Phe Ala Cys Cys Trp Asn Glu Trp Ala Pro Pro
930 935 940

CTA TTT AGC ATG AAG GAG CCC CAG GCA GGG TAT GCA CAG ACT GAC CAC 2880
Leu Phe Ser Met Lys Glu Pro Gln Ala Gly Tyr Ala Gln Thr Asp His
945 950 955 960

CAT CCC TCC CCA CCC AGG GTC CAC CCA ACC CGG TGA AGA GAC CAG GAG 2928
His Pro Ser Pro Pro Arg Val His Pro Thr Arg * Arg Asp Gln Glu
965 970 975

CAT TGT ACG CAT ACG CGG GTG GTA TTT TTA TGG ACC CCA ATC TGC AAT 2976
His Cys Thr His Thr Arg Val Val Phe Leu Trp Thr Pro Ile Cys Asn
980 985 990

TCC CAG ACA CCT GGG AAG TGG GAC ATT CTT TGT GTA TTT ATT TTC CTC 3024
Ser Gln Thr Pro Gly Lys Trp Asp Ile Leu Cys Val Phe Ile Phe Leu
995 1000 1005

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CCC AGG AGC TGG GGA GTG GTG GGG GGC TGC AGG TAC GGT TTA GCA TGT      3072
Pro Arg Ser Trp Gly Val Val Gly Gly Cys Arg Tyr Gly Leu Ala Cys
    1010                1015                1020

GTT TGG TTC TGG GGG TCT CTC CAG CCT TGT TTT GGG CCA AGT TGG AAC      3120
Val Trp Phe Trp Gly Ser Leu Gln Pro Cys Phe Gly Pro Ser Trp Asn
1025                1030                1035                1040

CTC TGG CCC TCC AGC TGG TGA CTA TGA ACT CCA GAC CCC TTC GTG CTC      3168
Leu Trp Pro Ser Ser Trp  *  Leu  *  Thr Pro Asp Pro Phe Val Leu
                1045                1050                1055

CCC GAC GCC TTC CCC TTG CAT CCT GTG TAA CCA TTT CGT TGG GCC CTC      3216
Pro Asp Ala Phe Pro Leu His Pro Val  *  Pro Phe Arg Trp Ala Leu
            1060                1065                1070

CCA AAA CCT ACA CAT AAA ACA TAC AGG AGG ACC ATT AAA TTG GC           3260
Pro Lys Pro Thr His Lys Thr Tyr Arg Arg Thr Ile Lys Leu
        1075                1080                1085
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1086 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Thr Ala Pro Ala Gly Ser Arg Arg Trp Ser Ala Ser Gln Phe Ser Ser
  1               5                  10                  15

Ala Gln His His Arg Ser Ala Glu Arg Thr Glu Leu Glu Ala Glu Arg
             20                  25                  30

 *  Arg Arg Arg Arg Ala Met Arg Arg Ala Gly Ala Ala Cys Ser Ala
         35                  40                  45

Met Asp Arg Leu Arg Leu Leu Leu Leu Leu Ile Leu Gly Val Ser Ser
     50                  55                  60

Gly Gly Ala Lys Glu Thr Cys Ser Thr Gly Leu Tyr Thr His Ser Gly
 65                  70                  75                      80

Glu Cys Cys Lys Ala Cys Asn Leu Gly Glu Gly Val Ala Gln Pro Cys
                 85                  90                  95

Gly Ala Asn Gln Thr Val Cys Glu Pro Cys Leu Asp Asn Val Thr Phe
            100                 105                 110

Ser Asp Val Val Ser Ala Thr Glu Pro Cys Lys Pro Cys Thr Glu Cys
        115                 120                 125

Leu Gly Leu Gln Ser Met Ser Ala Pro Cys Val Glu Ala Asp Asp Ala
    130                 135                 140

Val Cys Arg Cys Ala Tyr Gly Tyr Tyr Gln Asp Glu Glu Thr Gly His
145                 150                 155                     160

Cys Glu Ala Cys Ser Val Cys Glu Val Gly Ser Gly Leu Val Phe Ser
                165                 170                 175

Cys Gln Asp Lys Gln Asn Thr Val Cys Glu Glu Cys Pro Glu Gly Thr
            180                 185                 190

Tyr Ser Asp Glu Ala Asn His Val Asp Pro Cys Leu Pro Cys Thr Val
        195                 200                 205

Cys Glu Asp Thr Glu Arg Gln Leu Arg Glu Cys Thr Pro Trp Ala Asp
    210                 215                 220

Ala Glu Cys Glu Glu Ile Pro Gly Arg Trp Ile Pro Arg Ser Thr Pro
225                 230                 235                     240

Pro Glu Gly Ser Asp Ser Thr Ala Pro Ser Thr Gln Glu Pro Glu Val
                245                 250                 255

Pro Pro Glu Gln Asp Leu Val Pro Ser Thr Val Ala Asp Met Val Thr
            260                 265                 270

Thr Val Met Gly Ser Ser Gln Pro Val Val Thr Arg Gly Thr Thr Asp
        275                 280                 285

Asn Leu Ile Pro Val Tyr Cys Ser Ile Leu Ala Ala Val Val Val Gly
    290                 295                 300
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Leu Val Ala Tyr Ile Ala Phe Lys Arg Trp Asn Ser Cys Lys Gln Asn
305                 310                 315                 320

Lys Gln Gly Ala Asn Ser Arg Pro Val Asn Gln Thr Pro Pro Pro Glu
                325                 330                 335

Gly Glu Lys Leu His Ser Asp Ser Gly Ile Ser Val Asp Ser Gln Ser
            340                 345                 350

Leu His Asp Gln Gln Thr His Thr Gln Thr Ala Ser Gly Gln Ala Leu
        355                 360                 365

Lys Gly Asp Gly Asn Leu Tyr Ser Ser Leu Pro Leu Thr Lys Arg Glu
    370                 375                 380

Glu Val Glu Lys Leu Leu Asn Gly Asp Thr Trp Arg His Leu Ala Gly
385                 390                 395                 400

Glu Leu Gly Tyr Gln Pro Glu His Ile Asp Ser Phe Thr His Glu Ala
                405                 410                 415

Cys Pro Val Arg Ala Leu Leu Ala Ser Trp Gly Ala Gln Asp Ser Ala
            420                 425                 430

Thr Leu Asp Ala Leu Leu Ala Ala Leu Arg Arg Ile Gln Arg Ala Asp
        435                 440                 445

Ile Val Glu Ser Leu Cys Ser Glu Ser Thr Ala Thr Ser Pro Val  *
    450                 455                 460

Thr His Arg Leu Gly Ala Pro Val Leu Ser His Ile Pro Thr Thr Asp
465                 470                 475                 480

Val Leu Ala Ser Pro His Arg Ala Ala Pro Ser Pro Ser Gly Met Ala
                485                 490                 495

Gln Arg Ser Glu Arg Ser Ile Ser Val Gln Gly Leu Cys Val Pro Thr
            500                 505                 510

Pro Asp Ser Val Ala Ala Pro Glu Gly Ala Leu Ala Ser Asp His Pro
        515                 520                 525

Leu Leu Ser Lys Arg Glu Arg Glu Asp His Pro Ser Leu Thr Cys Ser
    530                 535                 540

Ile Ser Ile Ser Gly Leu Ser Phe Leu Ser Thr His  *  Leu Cys Gln
545                 550                 555                 560

Ile Trp Gly Phe Asp Thr Arg Arg Arg Glu Arg Gly His Pro  *  Asp
                565                 570                 575

Ser Gly Gly Thr Glu Glu Pro Glu Pro Trp Thr Pro His Cys Glu Pro
            580                 585                 590

Glu Asn Lys Gly Arg Gly Ile Val Val Gly  *  Thr Phe Leu Ser Pro
        595                 600                 605

Ser Leu Leu Pro Ser Gly Gln Arg Arg Gly Leu Arg Thr Tyr Leu Ser
    610                 615                 620

 *  Lys Gln Val Trp Asn Pro Ala His Thr Ser Leu Ser His Thr Gly
625                 630                 635                 640

Trp  *  Asn Pro Glu Lys Gly Arg Asp  *  Pro Arg Pro Pro Asn His
                645                 650                 655
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Arg Lys Asn Lys  *  Arg Leu Ile His Ser Val Ser Glu  *  Gly Arg
            660                 665                 670

Gln Val Cys Leu Leu Thr Gly Met Ala  *  Leu Ser Gly Lys Tyr Leu
        675                 680                 685

Glu Ala Met Ser Ala Pro Pro Ser Thr Thr Ser Arg Pro Leu Pro Asn
    690                 695                 700

Pro Cys Ala Asp Glu Leu Phe Val Gln Gly Leu Val His Trp Ser Ile
705                 710                 715                 720

Leu Met Glu Ser Ser  *  Gly Leu Arg Leu Ile His Lys Ala Phe Val
                725                 730                 735

Glu Arg  *  Ile Cys  *  Cys Ala His Ser Trp His Lys Pro Glu Ala
            740                 745                 750

Asn Thr Ala Leu Asn Val Ser Pro Arg Gly Gln Glu Pro Arg Thr Pro
        755                 760                 765

Thr Pro Gln Ser Asn Thr Ile Leu His Tyr Thr His Thr His Thr His
    770                 775                 780

Thr His Thr His Thr His Thr His Thr Asp Ile Leu Leu Phe Ser Pro
785                 790                 795                 800

Trp Leu Phe Trp Gly  *  Asp  *  Ile Leu Leu Gly Val Thr Ala Ser
                805                 810                 815

Glu Arg Ser Gly Gly Asp Arg Ala Glu Leu His Gly Ala Val Phe Leu
            820                 825                 830

Ala Pro Gly Ser Gly Arg Pro Arg Met Thr Ala Ser Glu Leu Val Ser
        835                 840                 845

Val Phe Gln Trp Pro Val Arg Gly Gly Asn Ala Pro Thr Pro Pro Leu
    850                 855                 860

Leu Glu Ala Ala Pro Arg Arg Leu Gln Cys Lys Arg Ala Asp Trp Cys
865                 870                 875                 880

Glu Asn Thr Arg Lys Ser Arg Cys Trp Pro Cys Ser Leu Trp Gln Leu
                885                 890                 895

Ser Pro Gln Leu Gln Gly Pro Cys Lys Gly Arg Ile Ser  *  Ala Arg
            900                 905                 910

Pro Gly Arg Gly Lys Arg Val Arg Phe Ser Gly Ala Phe Ser Arg Leu
        915                 920                 925

Leu Gly Leu Phe Cys Phe Ala Cys Cys Trp Asn Glu Trp Ala Pro Pro
    930                 935                 940

Leu Phe Ser Met Lys Glu Pro Gln Ala Gly Tyr Ala Gln Thr Asp His
945                 950                 955                 960

His Pro Ser Pro Pro Arg Val His Pro Thr Arg  *  Arg Asp Gln Glu
                965                 970                 975

His Cys Thr His Thr Arg Val Val Phe Leu Trp Thr Pro Ile Cys Asn
            980                 985                 990

Ser Gln Thr Pro Gly Lys Trp Asp Ile Leu Cys Val Phe Ile Phe Leu
        995                1000                1005
```

```
Pro Arg Ser Trp Gly Val Val Gly Gly Cys Arg Tyr Gly Leu Ala Cys
    1010                1015                1020

Val Trp Phe Trp Gly Ser Leu Gln Pro Cys Phe Gly Pro Ser Trp Asn
1025                1030                1035                1040

Leu Trp Pro Ser Ser Trp  *  Leu  *  Thr Pro Asp Pro Phe Val Leu
                1045                1050                1055

Pro Asp Ala Phe Pro Leu His Pro Val  *  Pro Phe Arg Trp Ala Leu
            1060                1065                1070

Pro Lys Pro Thr His Lys Thr Tyr Arg Arg Thr Ile Lys Leu
        1075                1080                1085
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 867 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..867

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ACA GCT CCG GCG GGC AGC AGG CGC TGG AGC GCA TCG CAG TTC AGC TCA        48
Thr Ala Pro Ala Gly Ser Arg Arg Trp Ser Ala Ser Gln Phe Ser Ser
  1               5                  10                  15

GCG CAG CAC CAT CGG TCT GCG GAG CGG ACT GAG CTA GAA GCG GAG CGC        96
Ala Gln His His Arg Ser Ala Glu Arg Thr Glu Leu Glu Ala Glu Arg
             20                  25                  30

TGA CGC CGG AGG CGT GCA ATG AGG AGG GCA GGT GCT GCC TGC AGC GCC       144
 *  Arg Arg Arg Arg Ala Met Arg Arg Ala Gly Ala Ala Cys Ser Ala
         35                  40                  45

ATG GAC CGG CTG CGC CTG CTG CTG CTG CTG ATT CTA GGG GTG TCC TCT       192
Met Asp Arg Leu Arg Leu Leu Leu Leu Leu Ile Leu Gly Val Ser Ser
     50                  55                  60

GGA GGT GCC AAG GAG ACA TGT TCC ACA GGC CTG TAC ACC CAC AGC GGA       240
Gly Gly Ala Lys Glu Thr Cys Ser Thr Gly Leu Tyr Thr His Ser Gly
 65                  70                  75                  80

GAG TGC TGC AAA GCC TGC AAC TTG GGC GAA GGC GTG GCC CAG CCC TGC       288
Glu Cys Cys Lys Ala Cys Asn Leu Gly Glu Gly Val Ala Gln Pro Cys
                 85                  90                  95

GGA GCC AAC CAG ACC GTG TGT GAA CCC TGC CTG GAC AAT GTT ACA TTC       336
Gly Ala Asn Gln Thr Val Cys Glu Pro Cys Leu Asp Asn Val Thr Phe
            100                 105                 110

TCC GAT GTG GTG AGC GCC ACT GAG CCG TGC AAG CCG TGC ACC GAG TGC       384
Ser Asp Val Val Ser Ala Thr Glu Pro Cys Lys Pro Cys Thr Glu Cys
        115                 120                 125
```

CTG GGC CTG CAG AGC ATG TCC GCT CCC TGT GTG GAG GCA GAC GAT GCA 432
Leu Gly Leu Gln Ser Met Ser Ala Pro Cys Val Glu Ala Asp Asp Ala
130 135 140

GTG TGC AGA TGT GCC TAT GGC TAC TAC CAG GAC GAG GAG ACT GGC CAC 480
Val Cys Arg Cys Ala Tyr Gly Tyr Tyr Gln Asp Glu Glu Thr Gly His
145 150 155 160

TGT GAG GCT TGC AGC GTG TGC GAG GTG GGC TCG GGA CTC GTG TTC TCC 528
Cys Glu Ala Cys Ser Val Cys Glu Val Gly Ser Gly Leu Val Phe Ser
165 170 175

TGC CAG GAC AAA CAG AAC ACA GTG TGT GAA GAG TGC CCA GAG GGC ACA 576
Cys Gln Asp Lys Gln Asn Thr Val Cys Glu Glu Cys Pro Glu Gly Thr
180 185 190

TAC TCA GAC GAA GCC AAC CAC GTG GAC CCG TGC CTA CCC TGC ACG GTG 624
Tyr Ser Asp Glu Ala Asn His Val Asp Pro Cys Leu Pro Cys Thr Val
195 200 205

TGC GAG GAC ACT GAG CGC CAG TTA CGC GAG TGC ACG CCC TGG GCT GAT 672
Cys Glu Asp Thr Glu Arg Gln Leu Arg Glu Cys Thr Pro Trp Ala Asp
210 215 220

GCT GAA TGC GAA GAG ATC CCT GGT CGA TGG ATC CCA AGG TCT ACG CCC 720
Ala Glu Cys Glu Glu Ile Pro Gly Arg Trp Ile Pro Arg Ser Thr Pro
225 230 235 240

CCG GAG GGC TCC GAC AGC ACA GCG CCC AGC ACC CAG GAG CCT GAG GTT 768
Pro Glu Gly Ser Asp Ser Thr Ala Pro Ser Thr Gln Glu Pro Glu Val
245 250 255

CCT CCA GAG CAA GAC CTT GTA CCC AGT ACA GTG GCG GAT ATG GTG ACC 816
Pro Pro Glu Gln Asp Leu Val Pro Ser Thr Val Ala Asp Met Val Thr
260 265 270

ACT GTG ATG GGC AGC TCC CAG CCT GTA GTG ACC CGC GGC ACC ACC GAC 864
Thr Val Met Gly Ser Ser Gln Pro Val Val Thr Arg Gly Thr Thr Asp
275 280 285

AAC 867
Asn

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 289 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Thr Ala Pro Ala Gly Ser Arg Arg Trp Ser Ala Ser Gln Phe Ser Ser
1 5 10 15

Ala Gln His His Arg Ser Ala Glu Arg Thr Glu Leu Glu Ala Glu Arg
20 25 30

* Arg Arg Arg Arg Ala Met Arg Arg Ala Gly Ala Ala Cys Ser Ala
35 40 45

```
Met Asp Arg Leu Arg Leu Leu Leu Leu Leu Ile Leu Gly Val Ser Ser
     50                  55                  60

Gly Gly Ala Lys Glu Thr Cys Ser Thr Gly Leu Tyr Thr His Ser Gly
 65                  70                  75                  80

Glu Cys Cys Lys Ala Cys Asn Leu Gly Glu Gly Val Ala Gln Pro Cys
                 85                  90                  95

Gly Ala Asn Gln Thr Val Cys Glu Pro Cys Leu Asp Asn Val Thr Phe
            100                 105                 110

Ser Asp Val Val Ser Ala Thr Glu Pro Cys Lys Pro Cys Thr Glu Cys
        115                 120                 125

Leu Gly Leu Gln Ser Met Ser Ala Pro Cys Val Glu Ala Asp Asp Ala
    130                 135                 140

Val Cys Arg Cys Ala Tyr Gly Tyr Tyr Gln Asp Glu Glu Thr Gly His
145                 150                 155                 160

Cys Glu Ala Cys Ser Val Cys Glu Val Gly Ser Gly Leu Val Phe Ser
                165                 170                 175

Cys Gln Asp Lys Gln Asn Thr Val Cys Glu Glu Cys Pro Glu Gly Thr
            180                 185                 190

Tyr Ser Asp Glu Ala Asn His Val Asp Pro Cys Leu Pro Cys Thr Val
        195                 200                 205

Cys Glu Asp Thr Glu Arg Gln Leu Arg Glu Cys Thr Pro Trp Ala Asp
    210                 215                 220

Ala Glu Cys Glu Glu Ile Pro Gly Arg Trp Ile Pro Arg Ser Thr Pro
225                 230                 235                 240

Pro Glu Gly Ser Asp Ser Thr Ala Pro Ser Thr Gln Glu Pro Glu Val
                245                 250                 255

Pro Pro Glu Gln Asp Leu Val Pro Ser Thr Val Ala Asp Met Val Thr
            260                 265                 270

Thr Val Met Gly Ser Ser Gln Pro Val Val Thr Arg Gly Thr Thr Asp
        275                 280                 285

Asn
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..66

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
CTC ATT CCT GTC TAT TGC TCC ATC TTG GCT GCT GTG GTC GTG GGC CTT        48
Leu Ile Pro Val Tyr Cys Ser Ile Leu Ala Ala Val Val Val Gly Leu
  1               5                  10                  15

GTG GCC TAT ATT GCT TTC                                                66
Val Ala Tyr Ile Ala Phe
             20
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Leu Ile Pro Val Tyr Cys Ser Ile Leu Ala Ala Val Val Val Gly Leu
  1               5                  10                  15

Val Ala Tyr Ile Ala Phe
             20
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 105 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..105

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
AAG AGG TGG AAC AGC TGC AAA CAA AAT AAA CAA GGC GCC AAC AGC CGC        48
Lys Arg Trp Asn Ser Cys Lys Gln Asn Lys Gln Gly Ala Asn Ser Arg
  1               5                  10                  15
```

```
CCC GTG AAC CAG ACG CCC CCA CCG GAG GGA GAG AAA CTG CAC AGC GAC      96
Pro Val Asn Gln Thr Pro Pro Pro Glu Gly Glu Lys Leu His Ser Asp
            20                  25                  30

AGT GGC ATC                                                          105
Ser Gly Ile
        35
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Lys Arg Trp Asn Ser Cys Lys Gln Asn Lys Gln Gly Ala Asn Ser Arg
  1               5                  10                  15

Pro Val Asn Gln Thr Pro Pro Pro Glu Gly Glu Lys Leu His Ser Asp
            20                  25                  30

Ser Gly Ile
        35
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2222 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..2222

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
TCT GTG GAC AGC CAG AGC CTG CAC GAC CAG CAG ACC CAT ACG CAG ACT      48
Ser Val Asp Ser Gln Ser Leu His Asp Gln Gln Thr His Thr Gln Thr
  1               5                  10                  15

GCC TCA GGC CAG GCC CTC AAG GGT GAT GGC AAC CTC TAC AGT AGC CTG      96
Ala Ser Gly Gln Ala Leu Lys Gly Asp Gly Asn Leu Tyr Ser Ser Leu
            20                  25                  30

CCC CTG ACC AAG CGT GAG GAG GTA GAG AAA CTG CTC AAC GGG GAT ACC     144
Pro Leu Thr Lys Arg Glu Glu Val Glu Lys Leu Leu Asn Gly Asp Thr
        35                  40                  45

TGG CGA CAT CTG GCA GGC GAG CTG GGT TAC CAG CCT GAA CAT ATA GAC     192
Trp Arg His Leu Ala Gly Glu Leu Gly Tyr Gln Pro Glu His Ile Asp
    50                  55                  60
```

```
TCC TTT ACC CAC GAG GCC TGC CCA GTG CGA GCC CTG CTG GCC AGC TGG      240
Ser Phe Thr His Glu Ala Cys Pro Val Arg Ala Leu Leu Ala Ser Trp
 65                 70                  75                  80

GGT GCC CAG GAC AGT GCA ACG CTT GAT GCC CTT TTA GCC GCC CTG CGA      288
Gly Ala Gln Asp Ser Ala Thr Leu Asp Ala Leu Leu Ala Ala Leu Arg
                85                  90                  95

CGC ATC CAG AGA GCT GAC ATT GTG GAG AGT CTA TGC AGC GAG TCC ACT      336
Arg Ile Gln Arg Ala Asp Ile Val Glu Ser Leu Cys Ser Glu Ser Thr
            100                 105                 110

GCC ACA TCC CCA GTG TGA ACT CAC AGA CTG GGA GCC CCT GTC CTG TCC      384
Ala Thr Ser Pro Val  *  Thr His Arg Leu Gly Ala Pro Val Leu Ser
        115                 120                 125

CAC ATT CCG ACG ACT GAT GTT CTA GCC AGC CCC CAC AGA GCT GCC CCC      432
His Ile Pro Thr Thr Asp Val Leu Ala Ser Pro His Arg Ala Ala Pro
    130                 135                 140

TCT CCC TCG GGG ATG GCC CAA CGG TCA GAA CGG AGC ATC TCT GTG CAG      480
Ser Pro Ser Gly Met Ala Gln Arg Ser Glu Arg Ser Ile Ser Val Gln
145                 150                 155                 160

GGC CTC TGT GTT CCC ACT CCT GAC TCC GTT GCT GCT CCC GAG GGG GCC      528
Gly Leu Cys Val Pro Thr Pro Asp Ser Val Ala Ala Pro Glu Gly Ala
                165                 170                 175

CTT GCT TCT GAC CAC CCT CTC CTC AGC AAG AGA GAG AGA GAG GAC CAC      576
Leu Ala Ser Asp His Pro Leu Leu Ser Lys Arg Glu Arg Glu Asp His
            180                 185                 190

CCG AGC CTG ACT TGC TCC ATT TCC ATC TCA GGC CTT TCC TTC CTT TCT      624
Pro Ser Leu Thr Cys Ser Ile Ser Ile Ser Gly Leu Ser Phe Leu Ser
        195                 200                 205

ACA CAT TAG CTG TGT CAG ATC TGG GGG TTT GAC ACT AGG AGA AGG GAG      672
Thr His  *  Leu Cys Gln Ile Trp Gly Phe Asp Thr Arg Arg Arg Glu
    210                 215                 220

CGG GGG CAC CCC TAA GAC TCA GGA GGT ACT GAA GAA CCA GAG CCA TGG      720
Arg Gly His Pro  *  Asp Ser Gly Gly Thr Glu Glu Pro Glu Pro Trp
225                 230                 235                 240

ACT CCA CAC TGT GAA CCG GAG AAC AAG GGG CGG GGC ATT GTG GTA GGC      768
Thr Pro His Cys Glu Pro Glu Asn Lys Gly Arg Gly Ile Val Val Gly
                245                 250                 255

TAG ACC TTC CTT AGC CCC TCC CTT CTC CCC TCT GGC CAA AGA AGA GGA      816
 *  Thr Phe Leu Ser Pro Ser Leu Leu Pro Ser Gly Gln Arg Arg Gly
            260                 265                 270

TTA CGG ACC TAT CTG AGC TGA AAG CAG GTT TGG AAC CCA GCC CAC ACT      864
Leu Arg Thr Tyr Leu Ser  *  Lys Gln Val Trp Asn Pro Ala His Thr
        275                 280                 285

TCT CTC TCA CAC ACA GGA TGG TAA AAC CCA GAG AAA GGC AGG GAC TGA      912
Ser Leu Ser His Thr Gly Trp  *  Asn Pro Glu Lys Gly Arg Asp  *
    290                 295                 300

CCT AGG CCA CCC AAC CAC AGG AAG AAC AAA TGA AGG CTG ATA CAC TCC      960
Pro Arg Pro Pro Asn His Arg Lys Asn Lys  *  Arg Leu Ile His Ser
305                 310                 315                 320

GTT TCT GAA TGA GGG CGT CAA GTG TGC TTG TTG ACA GGG ATG GCG TGA     1008
Val Ser Glu  *  Gly Arg Gln Val Cys Leu Leu Thr Gly Met Ala  *
                325                 330                 335
```

```
CTT TCA GGG AAA TAT CTG GAA GCC ATG TCT GCC CCG CCC TCA ACC ACT        1056
Leu Ser Gly Lys Tyr Leu Glu Ala Met Ser Ala Pro Pro Ser Thr Thr
            340                 345                 350

TCC AGG CCC CTA CCC AAC CCT TGT GCA GAT GAA CTG TTT GTT CAA GGG        1104
Ser Arg Pro Leu Pro Asn Pro Cys Ala Asp Glu Leu Phe Val Gln Gly
        355                 360                 365

CTG GTC CAT TGG TCT ATT CTG ATG GAG TCA AGC TAA GGG CTC AGG CTT        1152
Leu Val His Trp Ser Ile Leu Met Glu Ser Ser  *  Gly Leu Arg Leu
    370                 375                 380

ATC CAT AAG GCA TTT GTG GAG AGA TGA ATC TGT TAG TGC GCT CAT TCT        1200
Ile His Lys Ala Phe Val Glu Arg  *  Ile Cys  *  Cys Ala His Ser
385                 390                 395                 400

TGG CAT AAG CCT GAA GCC AAC ACG GCC CTT AAT GTC AGC CCT CGG GGT        1248
Trp His Lys Pro Glu Ala Asn Thr Ala Leu Asn Val Ser Pro Arg Gly
                405                 410                 415

CAG GAA CCA AGG ACT CCC ACC CCA CAA TCC AAC ACT ATA CTA CAT TAC        1296
Gln Glu Pro Arg Thr Pro Thr Pro Gln Ser Asn Thr Ile Leu His Tyr
            420                 425                 430

ACA CAC ACA CAC ACA CAC ACA CAC ACA CAC ACA CAC ACA CAC ACA GAT        1344
Thr His Thr His Thr His Thr His Thr His Thr His Thr His Thr Asp
        435                 440                 445

ATC TTG CTT TTC TCC CCA TGG CTC TTT TGG GGC TGA GAC TAG ATC CTG        1392
Ile Leu Leu Phe Ser Pro Trp Leu Phe Trp Gly  *  Asp  *  Ile Leu
    450                 455                 460

CTG GGA GTC ACT GCC AGT GAG AGA TCC GGA GGG GAC AGA GCT GAG CTT        1440
Leu Gly Val Thr Ala Ser Glu Arg Ser Gly Gly Asp Arg Ala Glu Leu
465                 470                 475                 480

CAT GGG GCT GTC TTC CTC GCC CCC GGG TCT GGC AGG CCA AGA ATG ACT        1488
His Gly Ala Val Phe Leu Ala Pro Gly Ser Gly Arg Pro Arg Met Thr
                485                 490                 495

GCA TCT GAG CTG GTG TCT GTC TTC CAA TGG CCT GTG CGT GGA GGA AAT        1536
Ala Ser Glu Leu Val Ser Val Phe Gln Trp Pro Val Arg Gly Gly Asn
            500                 505                 510

GCT CCC ACT CCT CCC CTT CTT GAA GCT GCC CCC AGA AGA CTA CAG TGC        1584
Ala Pro Thr Pro Pro Leu Leu Glu Ala Ala Pro Arg Arg Leu Gln Cys
        515                 520                 525

AAA AGA GCA GAC TGG TGT GAG AAC ACA AGA AAA AGC AGA TGC TGG CCC        1632
Lys Arg Ala Asp Trp Cys Glu Asn Thr Arg Lys Ser Arg Cys Trp Pro
    530                 535                 540

TGC AGT CTG TGG CAG CTT TCT CCT CAG CTT CAA GGC CCC TGC AAA GGA        1680
Cys Ser Leu Trp Gln Leu Ser Pro Gln Leu Gln Gly Pro Cys Lys Gly
545                 550                 555                 560

CGG ATT TCC TGA GCA CGG CCA GGA AGG GGC AAG AGG GTT CGG TTC AGT        1728
Arg Ile Ser  *  Ala Arg Pro Gly Arg Gly Lys Arg Val Arg Phe Ser
                565                 570                 575

GGC GCT TTC TCC CGG CTC CTT GGC CTG TTC TGT TTT GCT TGC TGT TGG        1776
Gly Ala Phe Ser Arg Leu Leu Gly Leu Phe Cys Phe Ala Cys Cys Trp
            580                 585                 590

AAT GAG TGG GCA CCC CCT CTA TTT AGC ATG AAG GAG CCC CAG GCA GGG        1824
Asn Glu Trp Ala Pro Pro Leu Phe Ser Met Lys Glu Pro Gln Ala Gly
        595                 600                 605
```

TAT GCA CAG ACT GAC CAC CAT CCC TCC CCA CCC AGG GTC CAC CCA ACC 1872
Tyr Ala Gln Thr Asp His His Pro Ser Pro Pro Arg Val His Pro Thr
610 615 620

CGG TGA AGA GAC CAG GAG CAT TGT ACG CAT ACG CGG GTG GTA TTT TTA 1920
Arg * Arg Asp Gln Glu His Cys Thr His Thr Arg Val Val Phe Leu
625 630 635 640

TGG ACC CCA ATC TGC AAT TCC CAG ACA CCT GGG AAG TGG GAC ATT CTT 1968
Trp Thr Pro Ile Cys Asn Ser Gln Thr Pro Gly Lys Trp Asp Ile Leu
645 650 655

TGT GTA TTT ATT TTC CTC CCC AGG AGC TGG GGA GTG GTG GGG GGC TGC 2016
Cys Val Phe Ile Phe Leu Pro Arg Ser Trp Gly Val Val Gly Gly Cys
660 665 670

AGG TAC GGT TTA GCA TGT GTT TGG TTC TGG GGG TCT CTC CAG CCT TGT 2064
Arg Tyr Gly Leu Ala Cys Val Trp Phe Trp Gly Ser Leu Gln Pro Cys
675 680 685

TTT GGG CCA AGT TGG AAC CTC TGG CCC TCC AGC TGG TGA CTA TGA ACT 2112
Phe Gly Pro Ser Trp Asn Leu Trp Pro Ser Ser Trp * Leu * Thr
690 695 700

CCA GAC CCC TTC GTG CTC CCC GAC GCC TTC CCC TTG CAT CCT GTG TAA 2160
Pro Asp Pro Phe Val Leu Pro Asp Ala Phe Pro Leu His Pro Val *
705 710 715 720

CCA TTT CGT TGG GCC CTC CCA AAA CCT ACA CAT AAA ACA TAC AGG AGG 2208
Pro Phe Arg Trp Ala Leu Pro Lys Pro Thr His Lys Thr Tyr Arg Arg
725 730 735

ACC ATT AAA TTG GC 2222
Thr Ile Lys Leu
740

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 740 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ser Val Asp Ser Gln Ser Leu His Asp Gln Gln Thr His Thr Gln Thr
1 5 10 15

Ala Ser Gly Gln Ala Leu Lys Gly Asp Gly Asn Leu Tyr Ser Ser Leu
20 25 30

Pro Leu Thr Lys Arg Glu Glu Val Glu Lys Leu Leu Asn Gly Asp Thr
35 40 45

Trp Arg His Leu Ala Gly Glu Leu Gly Tyr Gln Pro Glu His Ile Asp
50 55 60

Ser Phe Thr His Glu Ala Cys Pro Val Arg Ala Leu Leu Ala Ser Trp
65 70 75 80

Gly Ala Gln Asp Ser Ala Thr Leu Asp Ala Leu Leu Ala Ala Leu Arg
85 90 95

```
Arg Ile Gln Arg Ala Asp Ile Val Glu Ser Leu Cys Ser Glu Ser Thr
            100                 105                 110

Ala Thr Ser Pro Val  *  Thr His Arg Leu Gly Ala Pro Val Leu Ser
        115                 120                 125

His Ile Pro Thr Thr Asp Val Leu Ala Ser Pro His Arg Ala Ala Pro
    130                 135                 140

Ser Pro Ser Gly Met Ala Gln Arg Ser Glu Arg Ser Ile Ser Val Gln
145                 150                 155                 160

Gly Leu Cys Val Pro Thr Pro Asp Ser Val Ala Ala Pro Glu Gly Ala
                165                 170                 175

Leu Ala Ser Asp His Pro Leu Leu Ser Lys Arg Glu Arg Glu Asp His
            180                 185                 190

Pro Ser Leu Thr Cys Ser Ile Ser Ile Ser Gly Leu Ser Phe Leu Ser
        195                 200                 205

Thr His  *  Leu Cys Gln Ile Trp Gly Phe Asp Thr Arg Arg Arg Glu
    210                 215                 220

Arg Gly His Pro  *  Asp Ser Gly Gly Thr Glu Glu Pro Glu Pro Trp
225                 230                 235                 240

Thr Pro His Cys Glu Pro Glu Asn Lys Gly Arg Gly Ile Val Val Gly
                245                 250                 255

 *  Thr Phe Leu Ser Pro Ser Leu Leu Pro Ser Gly Gln Arg Arg Gly
            260                 265                 270

Leu Arg Thr Tyr Leu Ser  *  Lys Gln Val Trp Asn Pro Ala His Thr
        275                 280                 285

Ser Leu Ser His Thr Gly Trp  *  Asn Pro Glu Lys Gly Arg Asp  *
    290                 295                 300

Pro Arg Pro Pro Asn His Arg Lys Asn Lys  *  Arg Leu Ile His Ser
305                 310                 315                 320

Val Ser Glu  *  Gly Arg Gln Val Cys Leu Leu Thr Gly Met Ala  *
                325                 330                 335

Leu Ser Gly Lys Tyr Leu Glu Ala Met Ser Ala Pro Pro Ser Thr Thr
            340                 345                 350

Ser Arg Pro Leu Pro Asn Pro Cys Ala Asp Glu Leu Phe Val Gln Gly
        355                 360                 365

Leu Val His Trp Ser Ile Leu Met Glu Ser Ser  *  Gly Leu Arg Leu
    370                 375                 380

Ile His Lys Ala Phe Val Glu Arg  *  Ile Cys  *  Cys Ala His Ser
385                 390                 395                 400

Trp His Lys Pro Glu Ala Asn Thr Ala Leu Asn Val Ser Pro Arg Gly
                405                 410                 415

Gln Glu Pro Arg Thr Pro Thr Pro Gln Ser Asn Thr Ile Leu His Tyr
            420                 425                 430

Thr His Thr His Thr His Thr His Thr His Thr His Thr His Thr Asp
        435                 440                 445
```

```
Ile Leu Leu Phe Ser Pro Trp Leu Phe Trp Gly  *  Asp  *  Ile Leu
    450                 455                 460

Leu Gly Val Thr Ala Ser Glu Arg Ser Gly Gly Asp Arg Ala Glu Leu
465                 470                 475                 480

His Gly Ala Val Phe Leu Ala Pro Gly Ser Gly Arg Pro Arg Met Thr
                485                 490                 495

Ala Ser Glu Leu Val Ser Val Phe Gln Trp Pro Val Arg Gly Gly Asn
            500                 505                 510

Ala Pro Thr Pro Pro Leu Leu Glu Ala Ala Pro Arg Arg Leu Gln Cys
        515                 520                 525

Lys Arg Ala Asp Trp Cys Glu Asn Thr Arg Lys Ser Arg Cys Trp Pro
    530                 535                 540

Cys Ser Leu Trp Gln Leu Ser Pro Gln Leu Gln Gly Pro Cys Lys Gly
545                 550                 555                 560

Arg Ile Ser  *  Ala Arg Pro Gly Arg Gly Lys Arg Val Arg Phe Ser
                565                 570                 575

Gly Ala Phe Ser Arg Leu Leu Gly Leu Phe Cys Phe Ala Cys Cys Trp
            580                 585                 590

Asn Glu Trp Ala Pro Pro Leu Phe Ser Met Lys Glu Pro Gln Ala Gly
        595                 600                 605

Tyr Ala Gln Thr Asp His His Pro Ser Pro Pro Arg Val His Pro Thr
    610                 615                 620

Arg  *  Arg Asp Gln Glu His Cys Thr His Thr Arg Val Val Phe Leu
625                 630                 635                 640

Trp Thr Pro Ile Cys Asn Ser Gln Thr Pro Gly Lys Trp Asp Ile Leu
                645                 650                 655

Cys Val Phe Ile Phe Leu Pro Arg Ser Trp Gly Val Val Gly Gly Cys
            660                 665                 670

Arg Tyr Gly Leu Ala Cys Val Trp Phe Trp Gly Ser Leu Gln Pro Cys
        675                 680                 685

Phe Gly Pro Ser Trp Asn Leu Trp Pro Ser Ser Trp  *  Leu  *  Thr
    690                 695                 700

Pro Asp Pro Phe Val Leu Pro Asp Ala Phe Pro Leu His Pro Val  *
705                 710                 715                 720

Pro Phe Arg Trp Ala Leu Pro Lys Pro Thr His Lys Thr Tyr Arg Arg
                725                 730                 735

Thr Ile Lys Leu
            740
```

DATED this 7th day of October, 1998

THE WALTER AND ELIZA HALL INSTITUTE
OF MEDICAL RESEARCH
By Its Patent Attorneys
DAVIES COLLISON CAVE

FIG 1

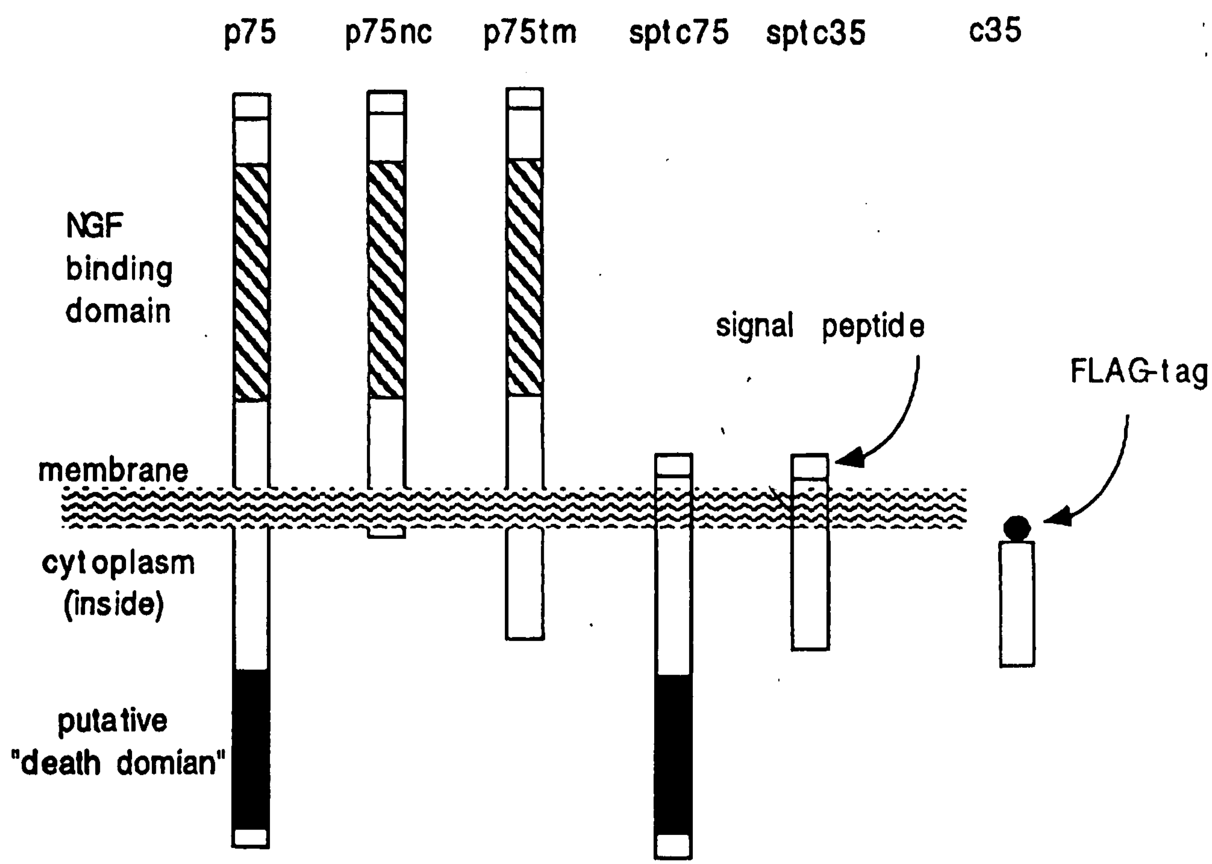
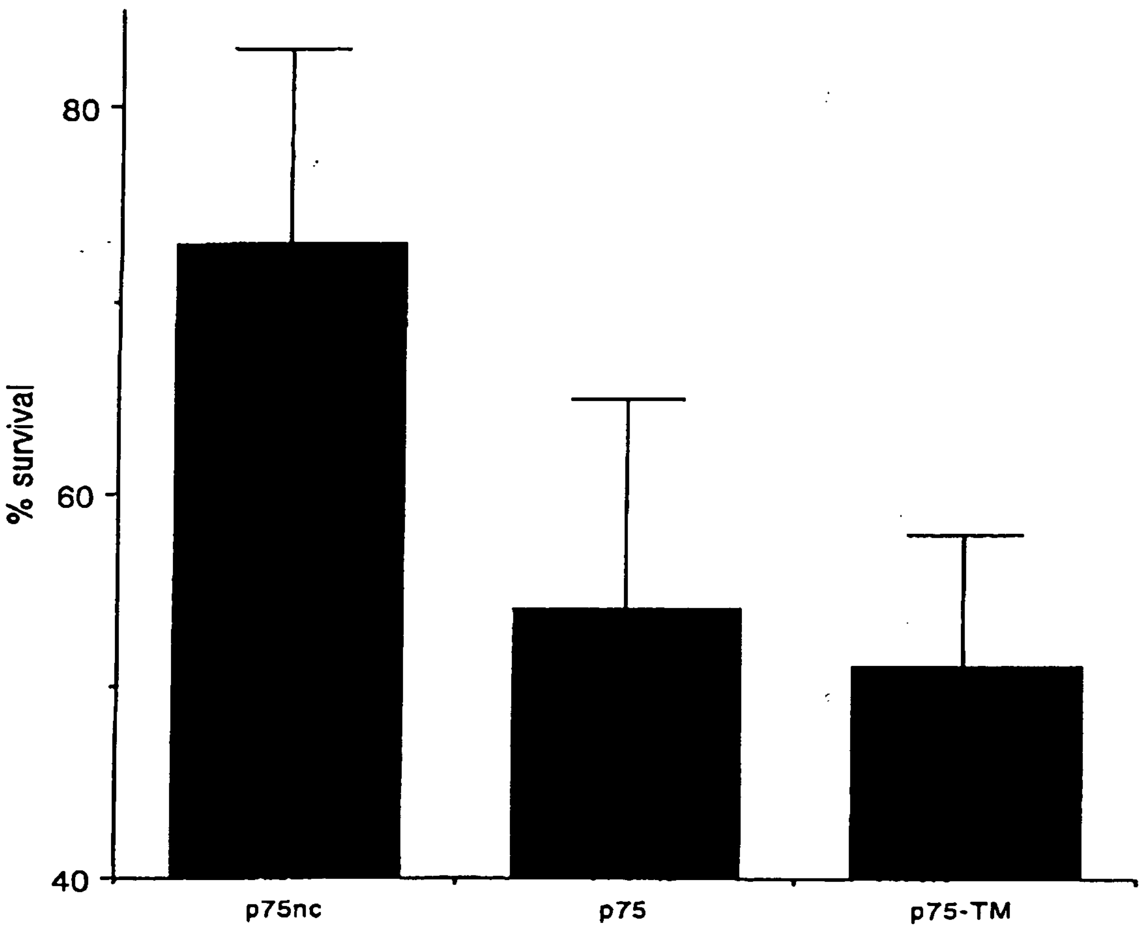

p75
p75nc
p75tm
sptc75
sptc35
c35
NGF binding domain
signal peptide
FLAG-tag
membrane
cytoplasm (inside)
putative "death domian"

FIG 2

Survival of DRG neurons 17 hrs after microinjection, cultured in LIF

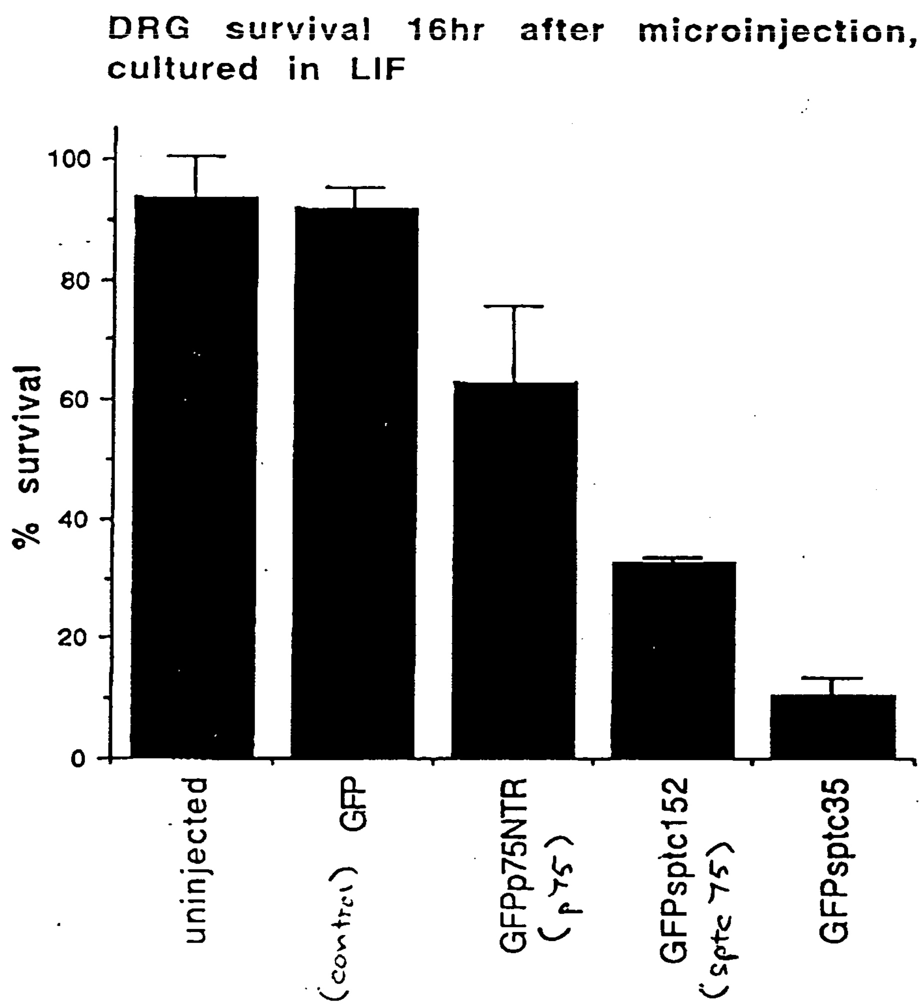


Expt S/U 11/11/97

p75nc.	p75	p75TM.
78.3±0.4	64.4±7	57.9±0.6.

expt S/U 4/11/97

FIG 3



GFP = the vector utilised

FIG 4

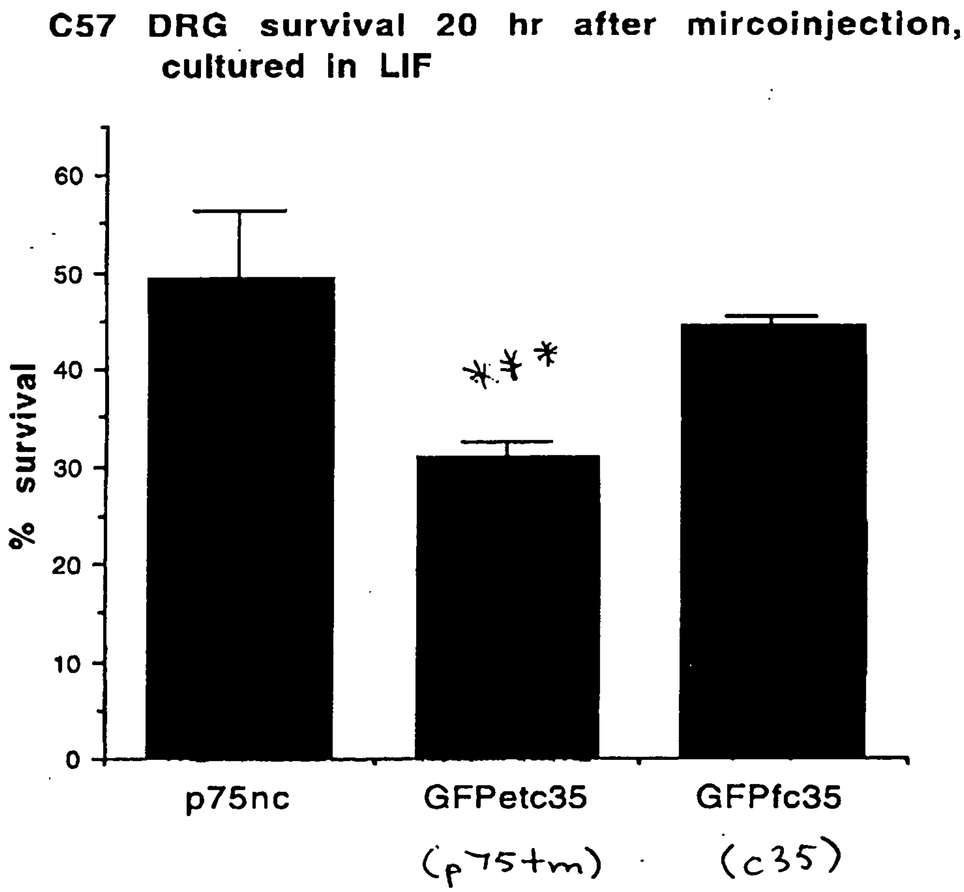
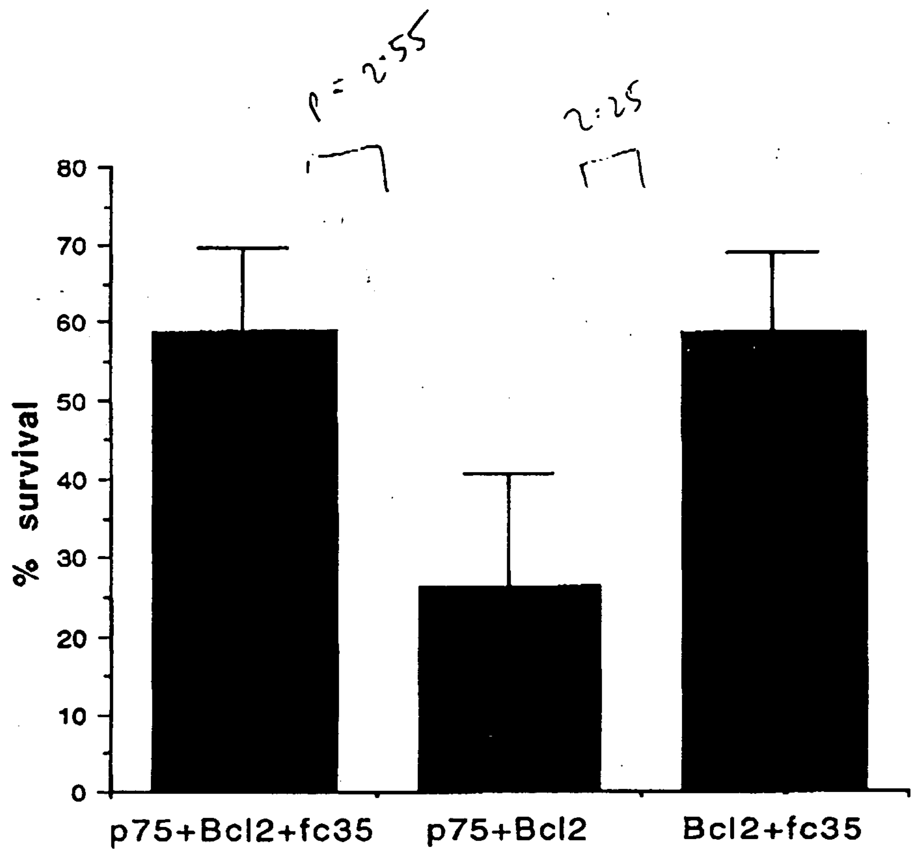

C57 DRG survival 20 hr after mircoinjection, cultured in LIF
% survival
0
10
20
30
40
50
60
***
p75nc
GFPetc35
(p75+m)
GFPfc35
(c35)

Fig 5.



p = 2:55
2:25
% survival
80
70
60
50
40
30
20
10
0
p75+Bcl2+fc35
p75+Bcl2
Bcl2+fc35